SDS – POLYACRYLAMIDE GEL OF OSTEOINDUCTIVELY ACTIVE PROTEINS FROM HPLC

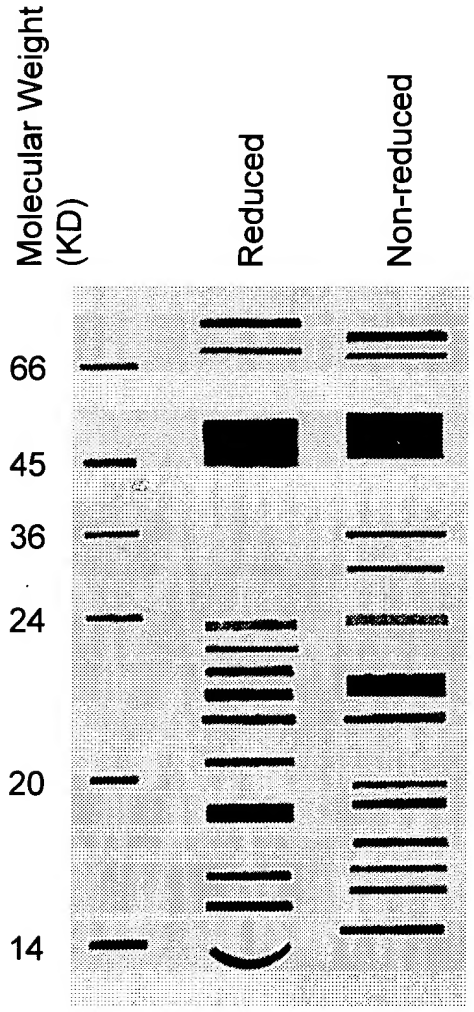


FIG. 1

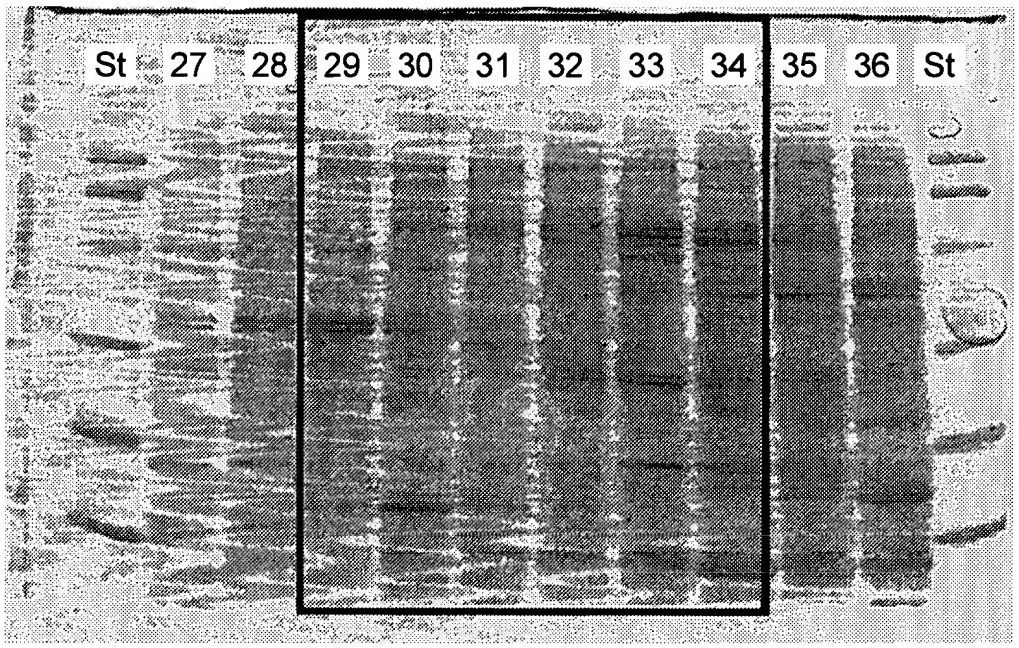

St
27
28
29
30
31
32
33
34
35
36
St

FIG.2

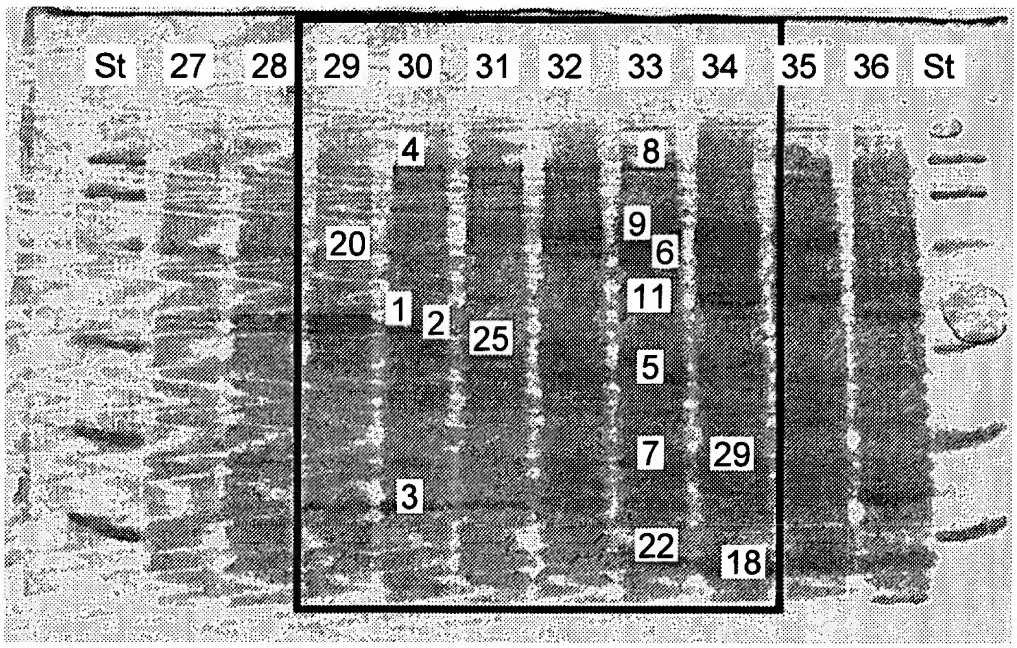
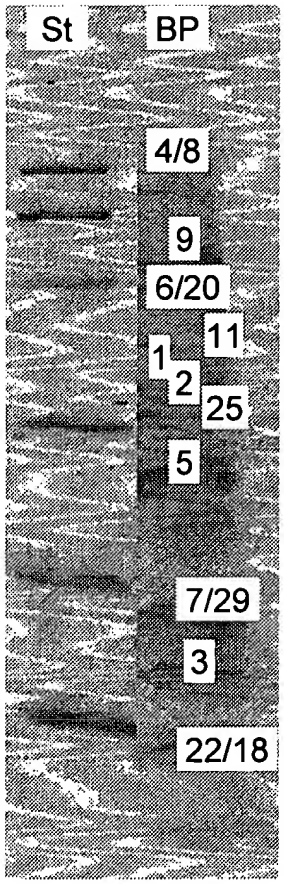

St
27
28
29
30
31
32
33
34
35
36
St
4
8
9
6
20
11
1
2
25
5
7
29
3
22
18

FIG.3



Band No.	Identity
1	Histone H1.c
2	Histone H1.c
3	Ribosomal protein RS20
4	Similar to ribosomal protein LORP
5	BMP-3
6	α2 macroglobulin RAP and BMP-3
7	Similar to ribosomal protein LORP
8	BMP-3
9	BMP-3
11	Ribosomal protein RL6 and BMP-3
18	TGF-β2/SPP24
20	Factor H
22	TGF-β2
25	BMP-3 and H1.x
29	BMP-3 and ribosomal protein RL32

FIG.4

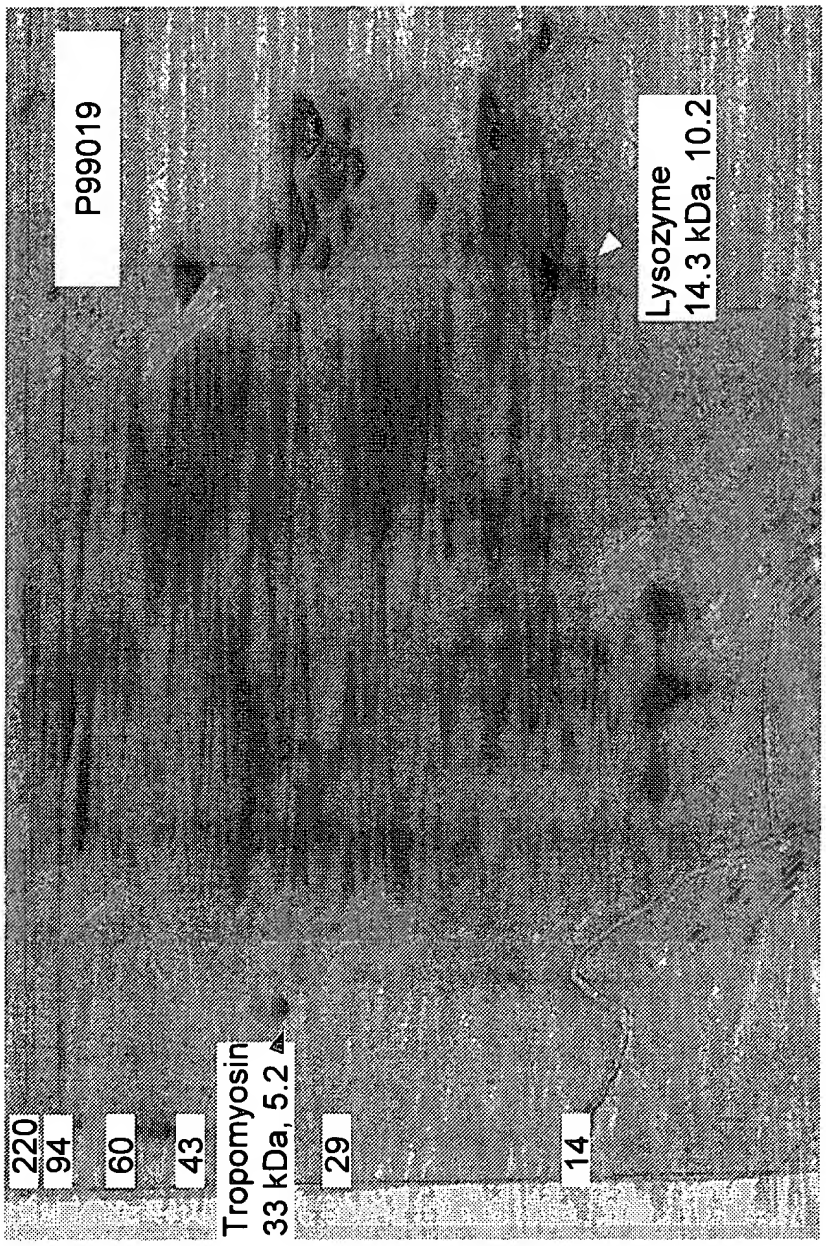

P99019
Lysozyme
14.3 kDa, 10.2
Tropomyosin
33 kDa, 5.2
220
94
60
43
29
14

FIG. 5

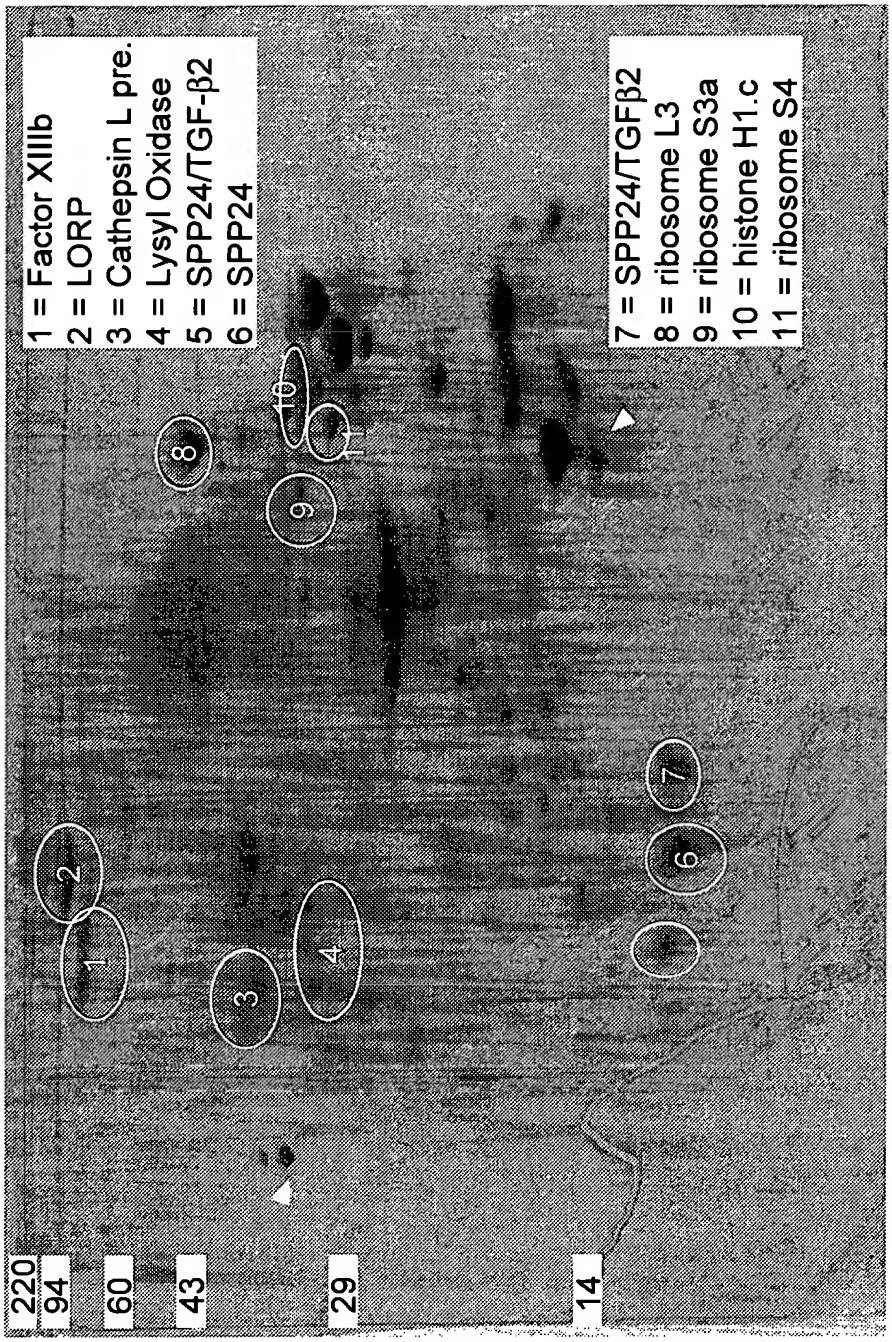

1 = Factor XIIIb
2 = LORP
3 = Cathepsin L pre.
4 = Lysyl Oxidase
5 = SPP24/TGF-β2
6 = SPP24
7 = SPP24/TGFβ2
8 = ribosome L3
9 = ribosome S3a
10 = histone H1.c
11 = ribosome S4
220
94
60
43
29
14

FIG.6

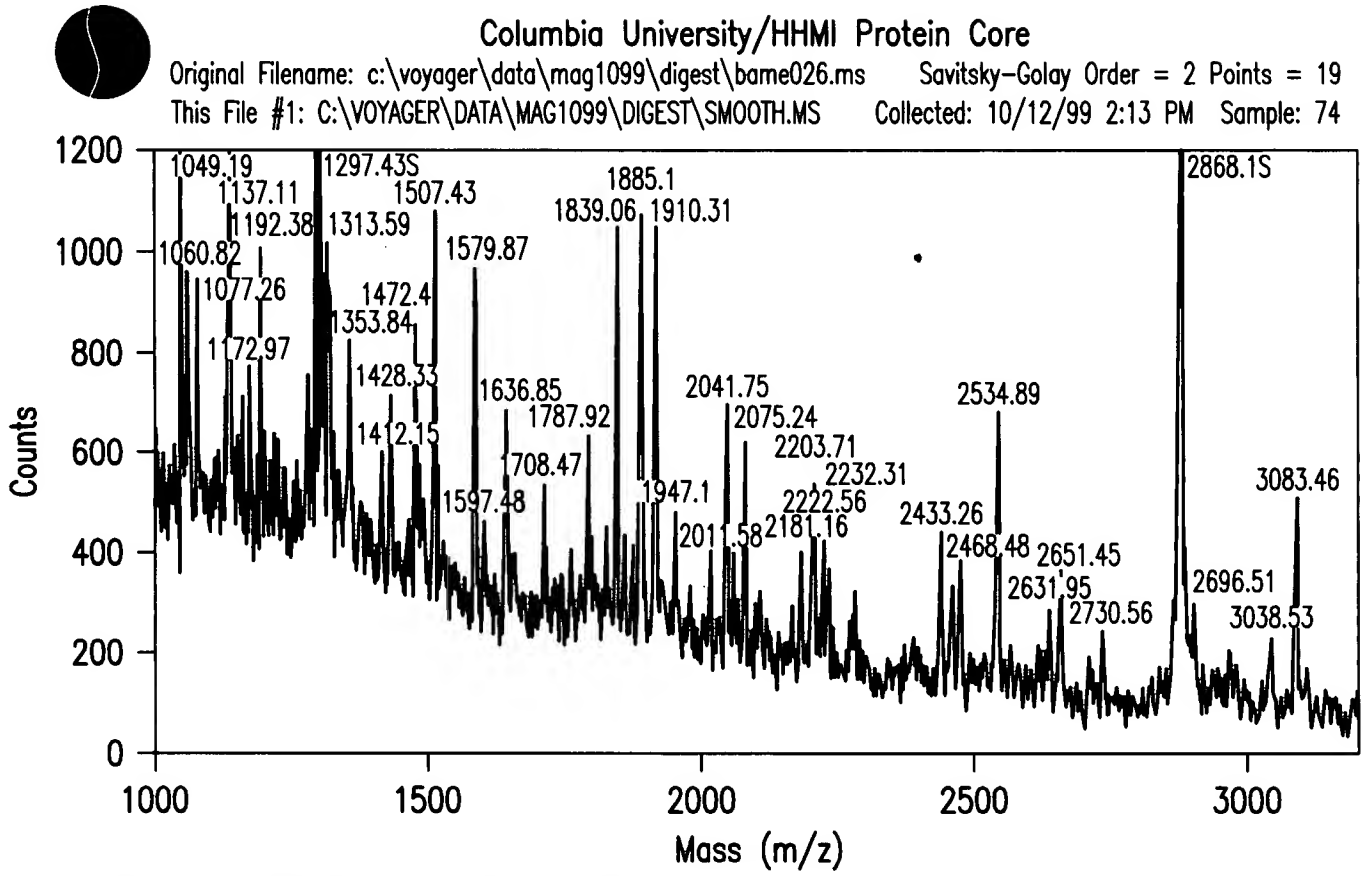

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1099\digest\bame026.ms Savitsky-Golay Order = 2 Points = 19
This File #1: C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS Collected: 10/12/99 2:13 PM Sample: 74
Counts
Mass (m/z)
1049.19
1137.11
1192.38
1060.82
1077.26
1172.97
1297.43S
1313.59
1353.84
1472.4
1428.33
1412.15
1507.43
1579.87
1597.48
1636.85
1787.92
1708.47
1839.06
1885.1
1910.31
1947.1
2011.58
2041.75
2075.24
2203.71
2222.56
2181.16
2232.31
2433.26
2534.89
2468.48
2651.45
2631.95
2730.56
2696.51
3038.53
3083.46
2868.1S
Comment: BARNES-1, trypsin, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 20000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.075 %
Delay: 50 ON
Laser: 1965
Scans Averaged: 256
Pressure: 1.70e-06
Low Mass Gate: 500.0
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7A

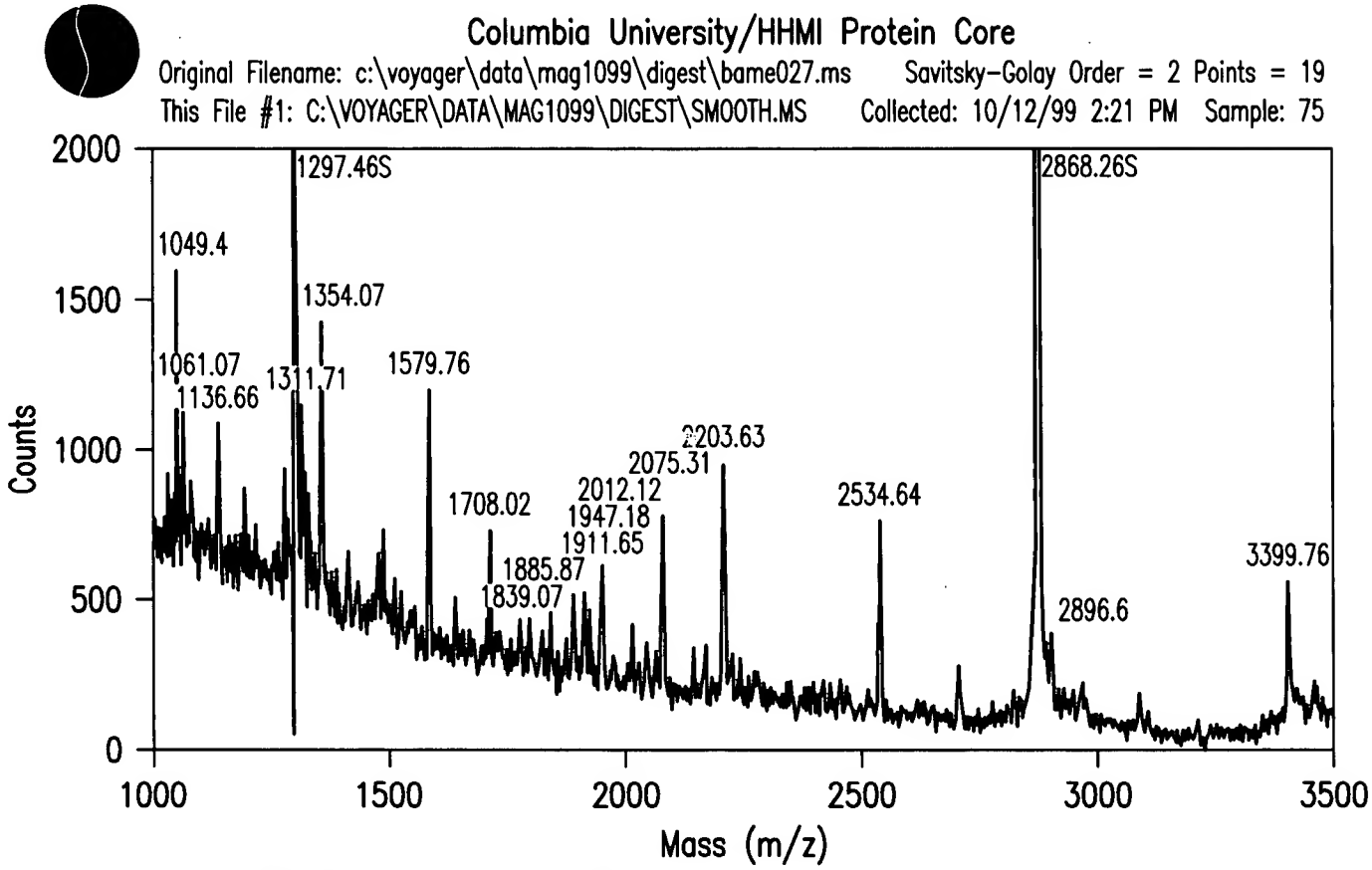

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1099\digest\bame027.ms Savitsky-Golay Order = 2 Points = 19
This File #1: C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS Collected: 10/12/99 2:21 PM Sample: 75
Counts
Mass (m/z)
1297.46S
2868.26S
1049.4
1354.07
1061.07
1136.66
1311.71
1579.76
2203.63
2075.31
2012.12
1947.18
1911.65
1708.02
1885.87
1839.07
2534.64
3399.76
2896.6
Comment: BARNES-2, trypsin, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 20000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.075 %
Delay: 50 ON
Laser: 1965
Scans Averaged: 256
Pressure: 1.58e-06
Low Mass Gate: 500.0
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7B

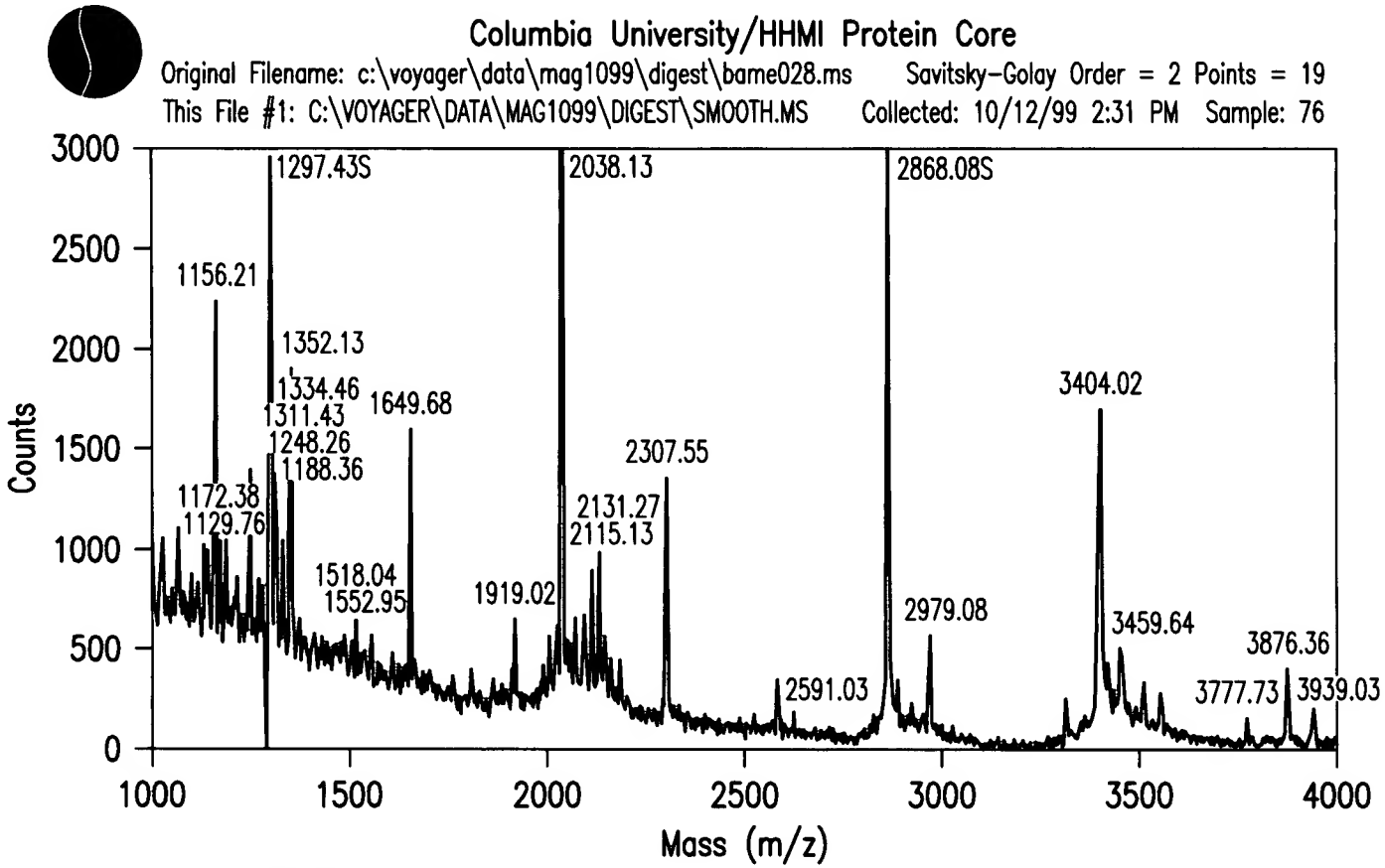

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1099\digest\barne028.ms Savitsky-Golay Order = 2 Points = 19
This File #1: C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS Collected: 10/12/99 2:31 PM Sample: 76
Counts
Mass (m/z)
1297.43S
2038.13
2868.08S
1156.21
1352.13
1334.46
1311.43
1649.68
1248.26
1188.36
1172.38
1129.76
2307.55
2131.27
2115.13
3404.02
1518.04
1552.95
1919.02
2979.08
3459.64
3876.36
2591.03
3777.73
3939.03
Comment: BARNES-3, trypsin, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 20000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.075 %
Delay: 50 ON
Laser: 1965
Scans Averaged: 256
Pressure: 1.5e-06
Low Mass Gate: 500.0
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7C

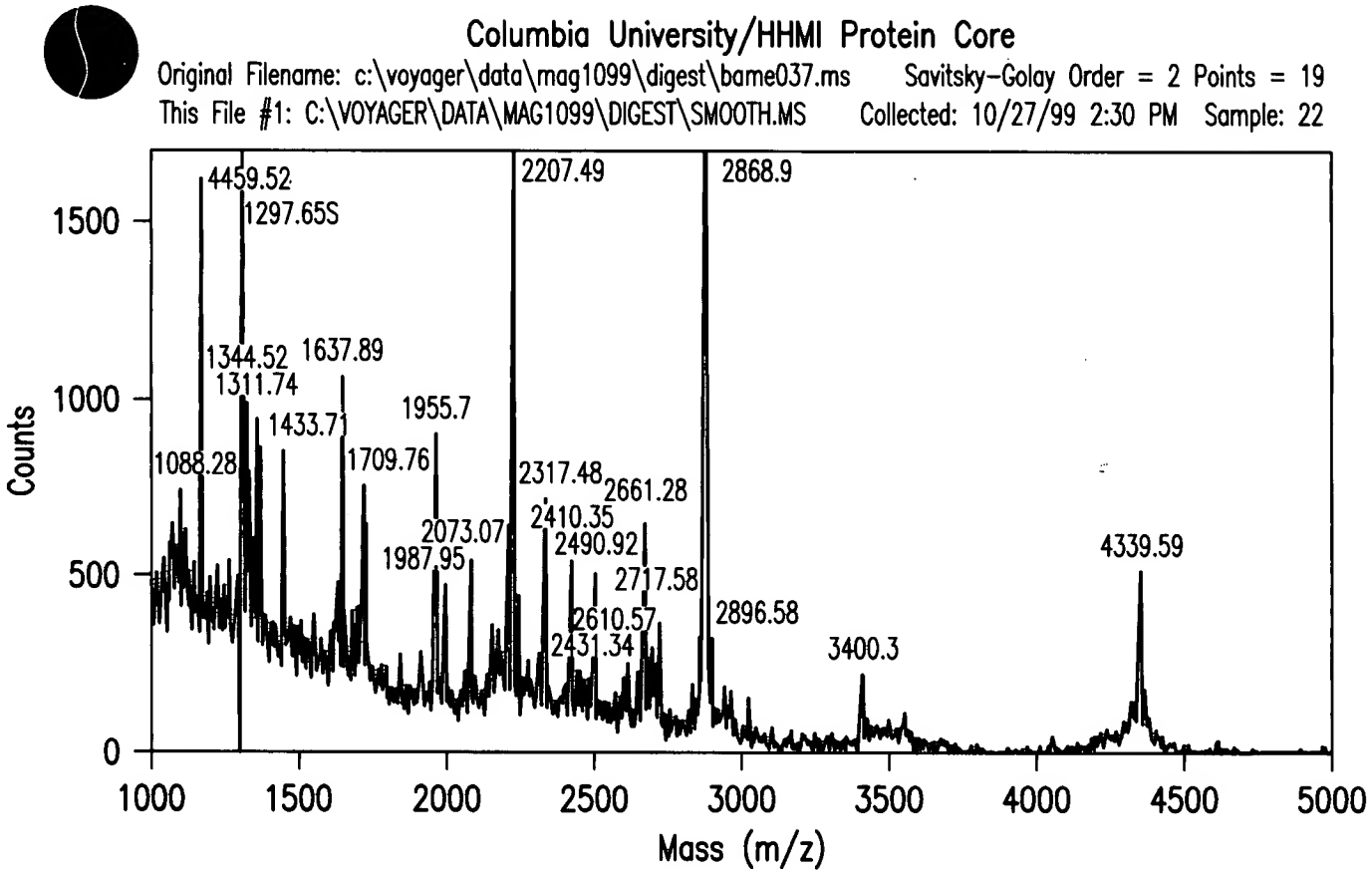
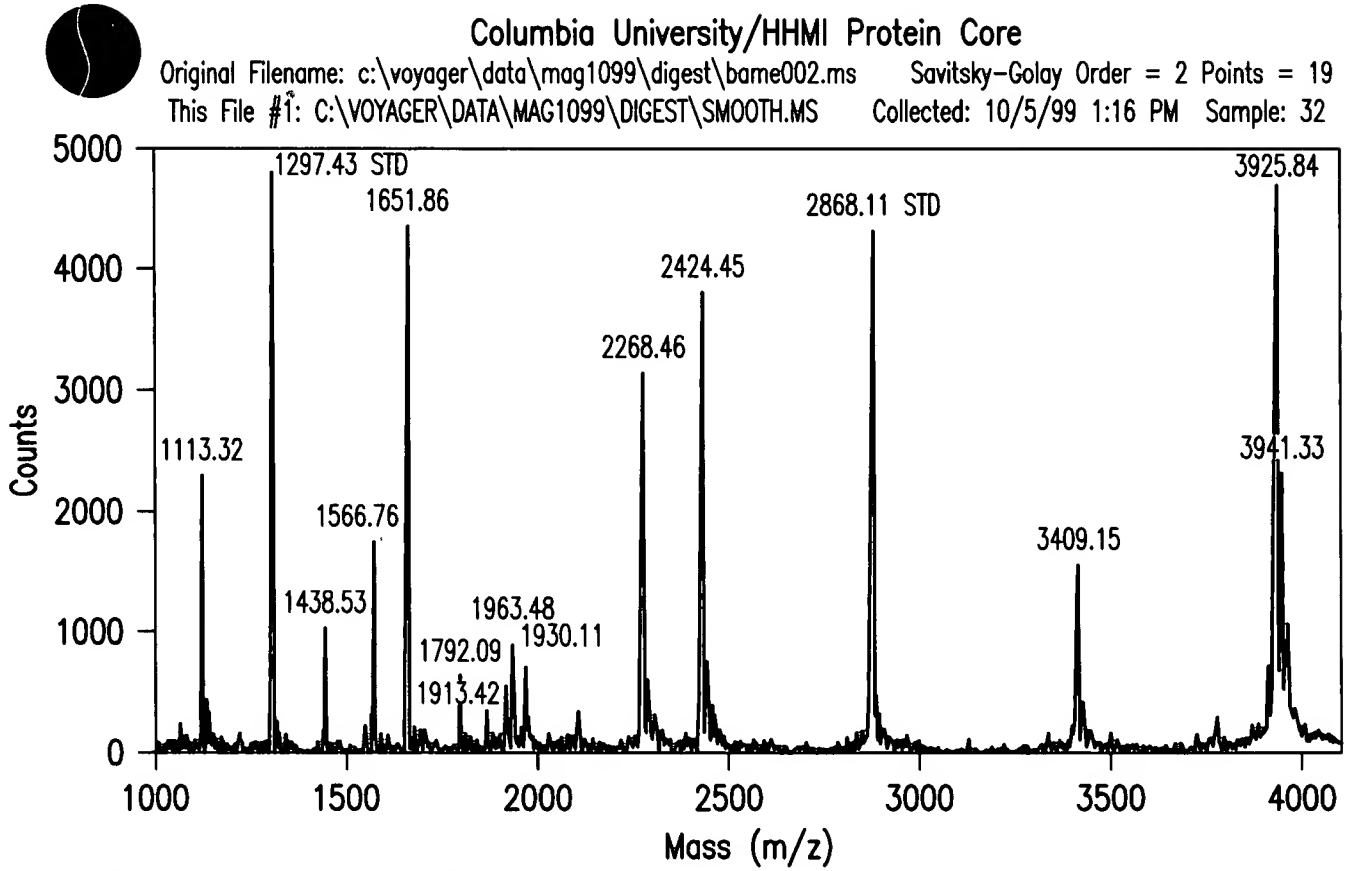

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1099\digest\barne037.ms Savitsky-Golay Order = 2 Points = 19
This File #1: C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS Collected: 10/27/99 2:30 PM Sample: 22
4459.52
1297.65S
2207.49
2868.9
1344.52
1637.89
1311.74
1433.71
1955.7
1088.28
1709.76
2317.48
2661.28
2410.35
2073.07
2490.92
1987.95
4339.59
2717.58
2610.57
2896.58
2431.34
3400.3
Counts
0
500
1000
1500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Mass (m/z)
Comment: barnes-4, trys 5 % S=std.
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 20000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.075 %
Delay: 50 ON
Laser: 1965
Scans Averaged: 256
Pressure: 9.04e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7D



Comment: barnes tryps #5

Method: LDE1000A	Accelerating Voltage: 20000	Laser: 1965	Mirror Ratio: 1.060
Mode: Linear	Grid Voltage: 94.000 %	Scans Averaged: 121	PSD Mirror Ratio:
	Guide Wire Voltage: 0.075 %	Pressure: 3.68e-07	Timed Ion Selector: 16.1 OFF
	Delay: 50 ON	Low Mass Gate: 500.0	Negative Ions: OFF

FIG.7E

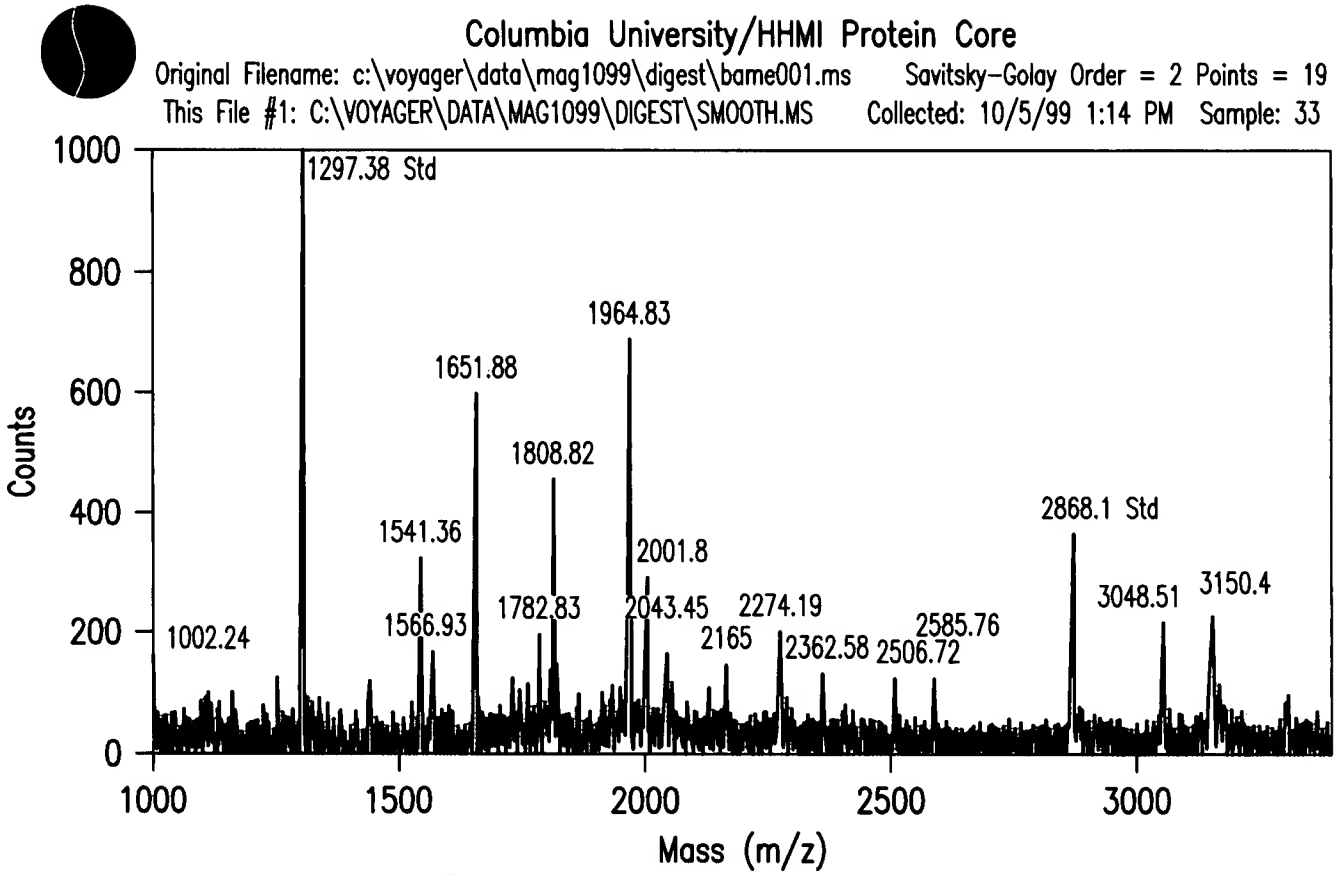

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1099\digest\barne001.ms Savitsky-Golay Order = 2 Points = 19
This File #1: C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS Collected: 10/5/99 1:14 PM Sample: 33
Counts
Mass (m/z)
1297.38 Std
1964.83
1651.88
1808.82
1541.36
2001.8
2868.1 Std
3048.51
3150.4
1002.24
1566.93
1782.83
2043.45
2274.19
2165
2362.58
2506.72
2585.76
Comment: barnes tryps #6
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 20000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.075 %
Delay: 50 ON
Laser: 1965
Scans Averaged: 256
Pressure: 4.06e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7F

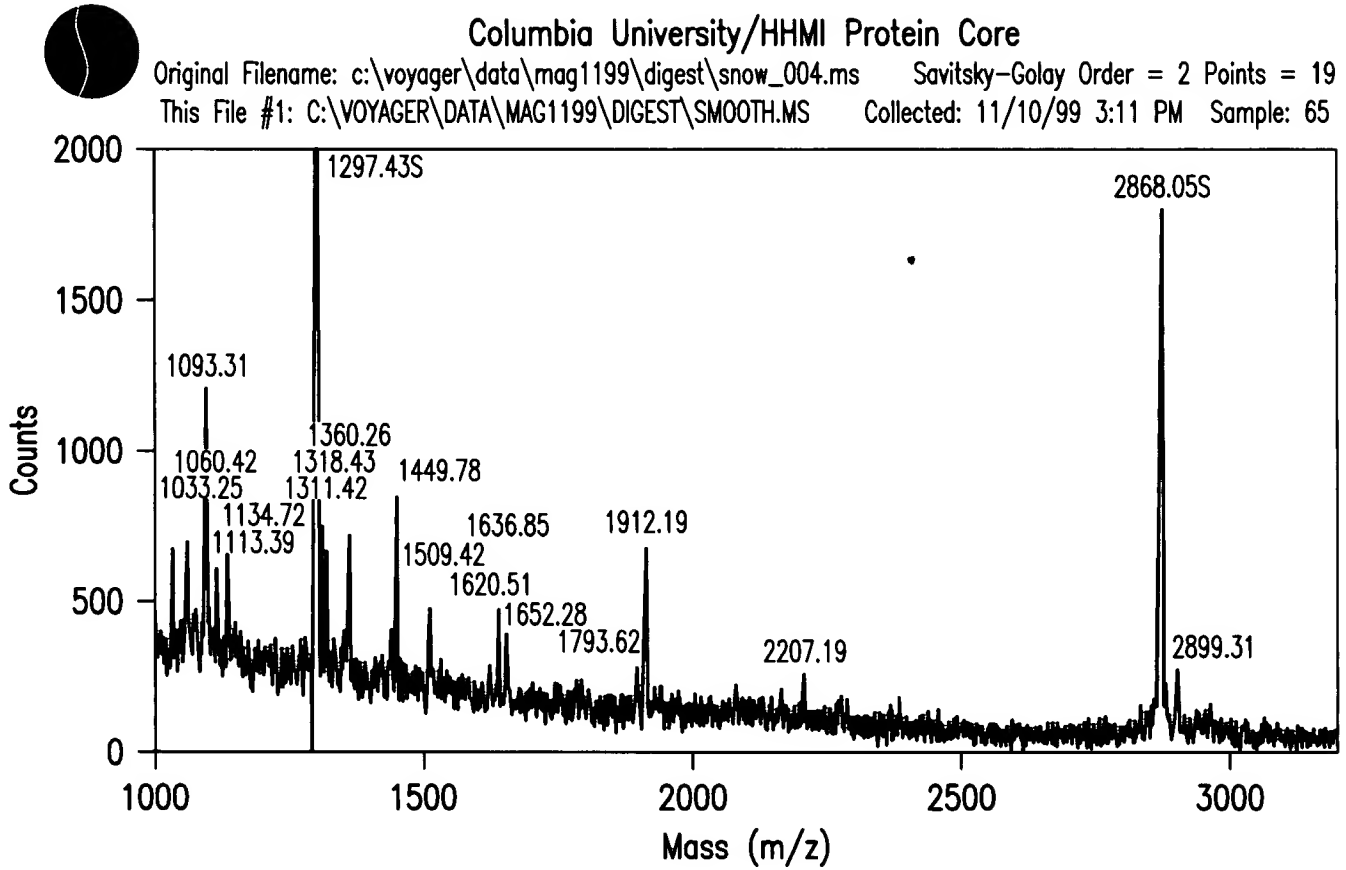

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1199\digest\snow_004.ms Savitsky-Golay Order = 2 Points = 19
This File #1: C:\VOYAGER\DATA\MAG1199\DIGEST\SMOOTH.MS Collected: 11/10/99 3:11 PM Sample: 65
Counts
Mass (m/z)
1297.43S
2868.05S
1093.31
1360.26
1060.42
1318.43
1449.78
1033.25
1311.42
1134.72
1636.85
1912.19
1113.39
1509.42
1620.51
1652.28
1793.62
2207.19
2899.31
Comment: barnes-7, tryp, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 20000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.075 %
Delay: 50 ON
Laser: 1965
Scans Averaged: 256
Pressure: 5.89e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7G

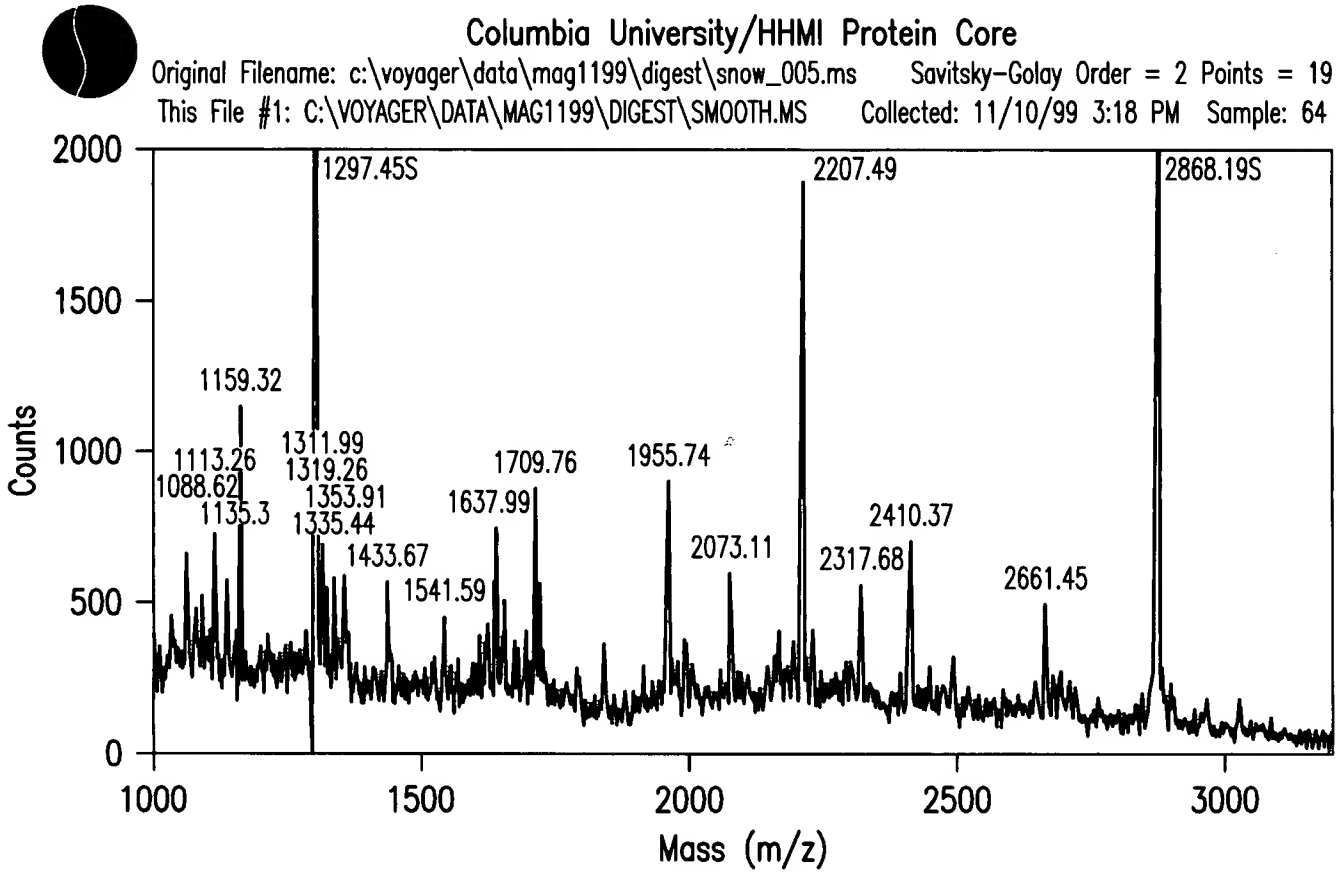

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1199\digest\snow_005.ms Savitsky-Golay Order = 2 Points = 19
This File #1: C:\VOYAGER\DATA\MAG1199\DIGEST\SMOOTH.MS Collected: 11/10/99 3:18 PM Sample: 64
Counts
Mass (m/z)
1297.45S
2207.49
2868.19S
1159.32
1311.99
1319.26
1113.26
1088.62
1135.3
1353.91
1335.44
1637.99
1709.76
1955.74
2073.11
2410.37
2317.68
2661.45
1433.67
1541.59
Comment: barnes-8, tryp, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 20000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.075 %
Delay: 50 ON
Laser: 1965
Scans Averaged: 256
Pressure: 4.01e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.060
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7H

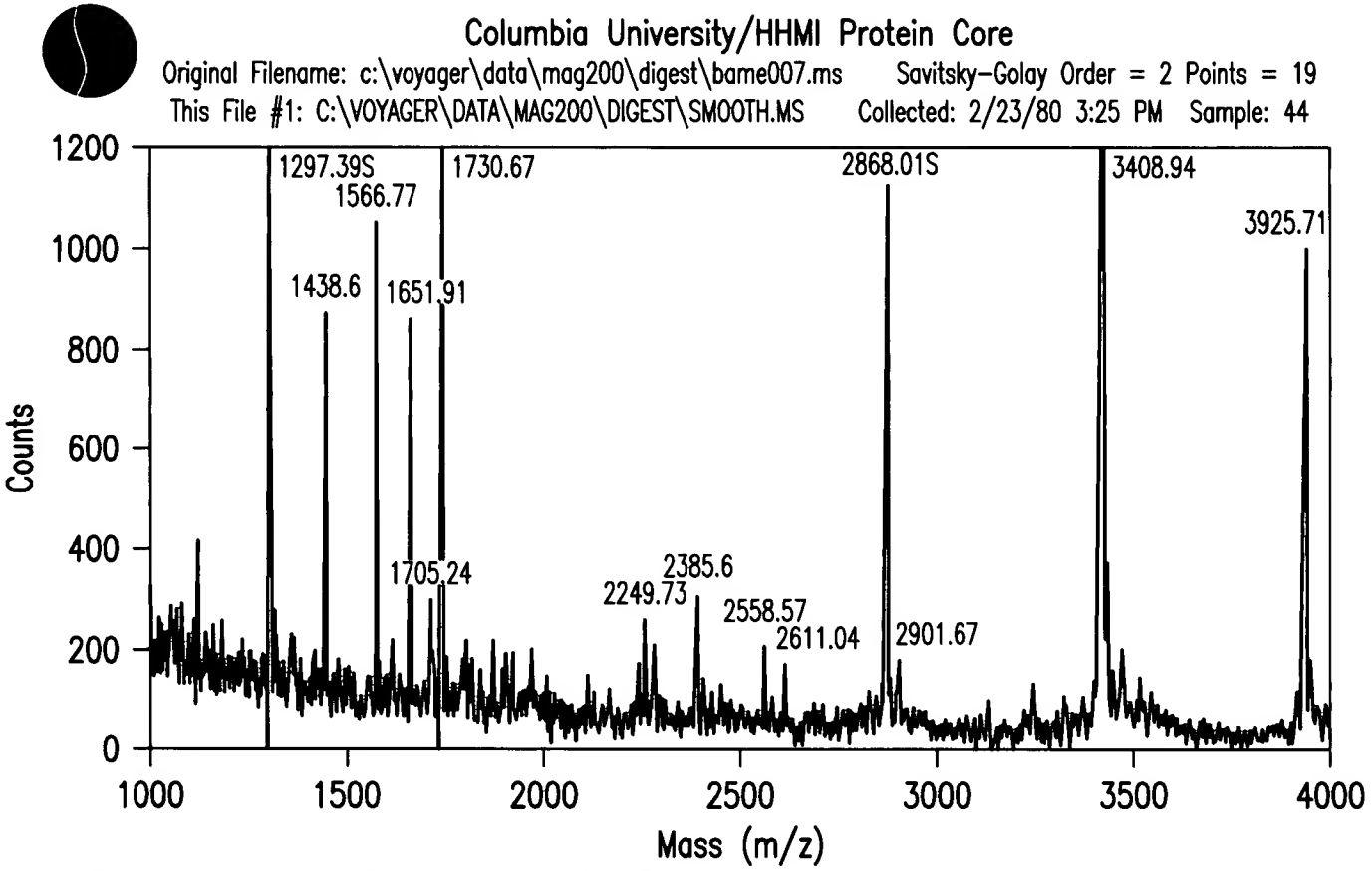


FIG. 7I

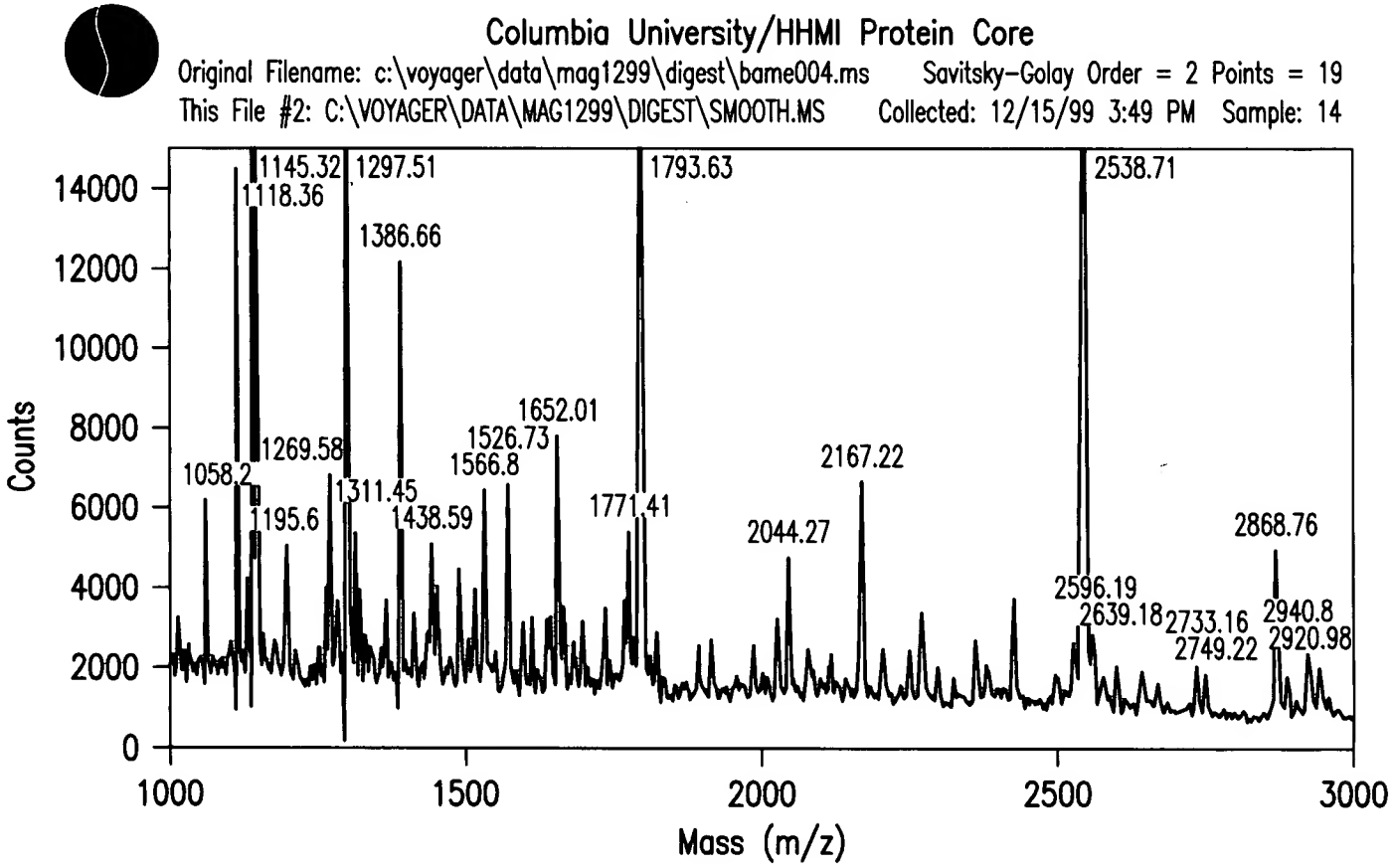

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1299\digest\barne004.ms Savitsky-Golay Order = 2 Points = 19
This File #2: C:\VOYAGER\DATA\MAG1299\DIGEST\SMOOTH.MS Collected: 12/15/99 3:49 PM Sample: 14
Counts
Mass (m/z)
1145.32
1118.36
1297.51
1386.66
1793.63
2538.71
1652.01
1526.73
1566.8
1269.58
1058.2
1311.45
1195.6
1438.59
1771.41
2044.27
2167.22
2868.76
2596.19
2639.18
2733.16
2749.22
2940.8
2920.98
Comment: barnes-11, tryp, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.090 %
Delay: 50 ON
Laser: 1965
Scans Averaged: 256
Pressure: 5.84e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.080
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7J

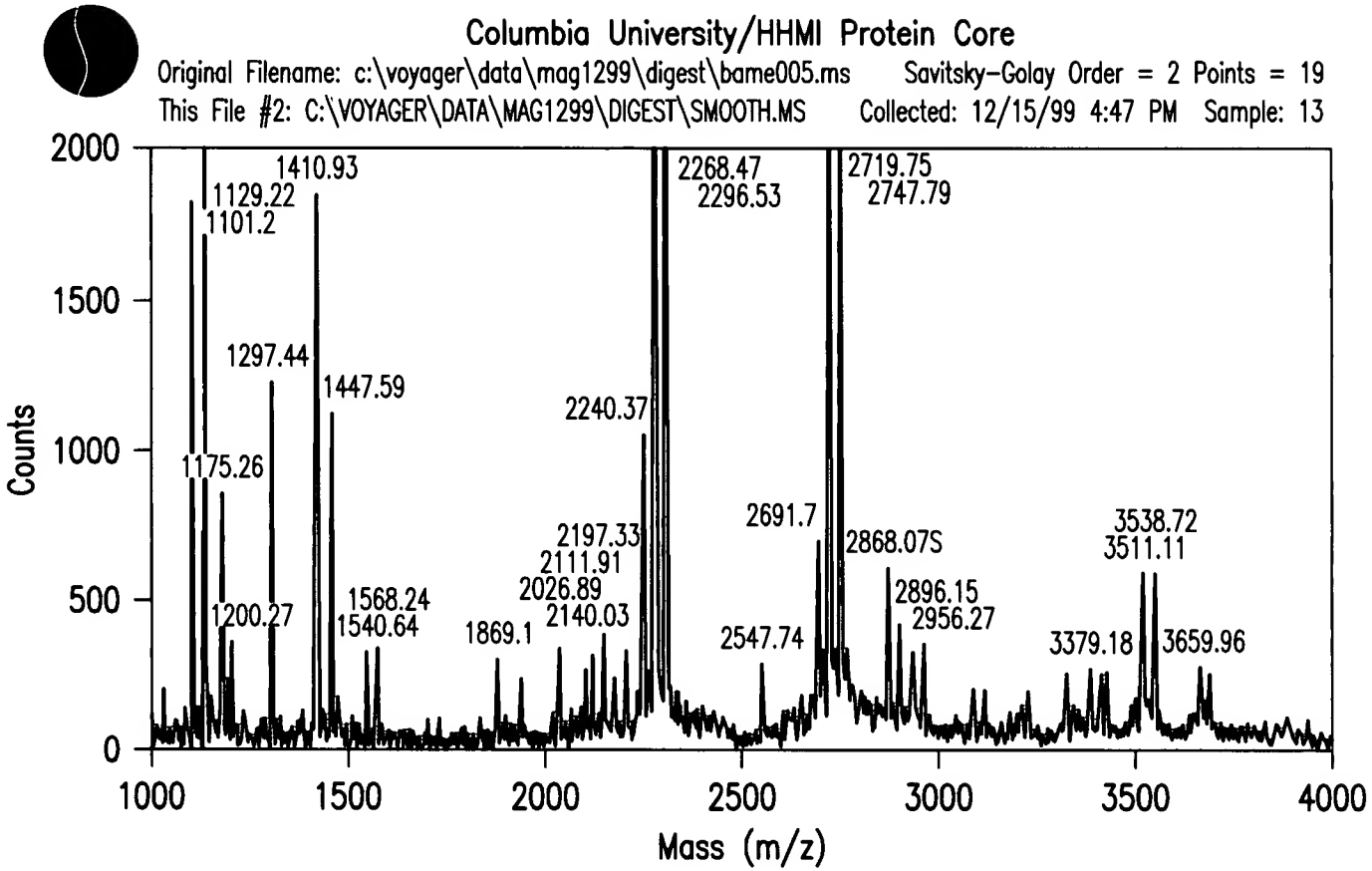

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag1299\digest\barne005.ms Savitsky-Golay Order = 2 Points = 19
This File #2: C:\VOYAGER\DATA\MAG1299\DIGEST\SMOOTH.MS Collected: 12/15/99 4:47 PM Sample: 13
Counts
Mass (m/z)
1410.93
1129.22
1101.2
1297.44
1447.59
1175.26
1200.27
1568.24
1540.64
1869.1
2240.37
2197.33
2111.91
2026.89
2140.03
2268.47
2296.53
2691.7
2547.74
2719.75
2747.79
2868.07S
2896.15
2956.27
3538.72
3511.11
3379.18
3659.96
Comment: barnes-18, tryp, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.090 %
Delay: 50 ON
Laser: 1745
Scans Averaged: 256
Pressure: 2.90e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.080
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7K

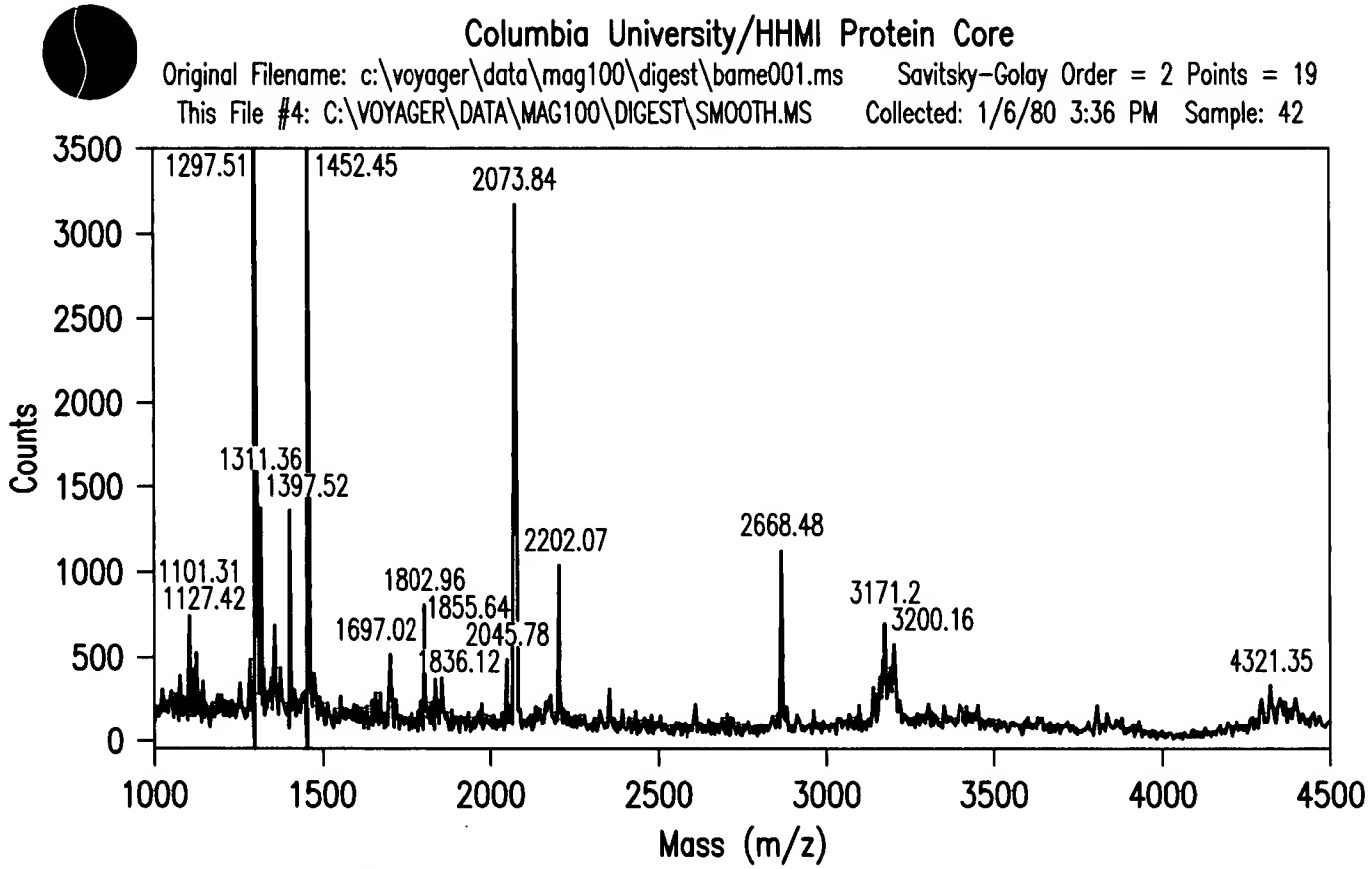

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag100\digest\barne001.ms Savitsky-Golay Order = 2 Points = 19
This File #4: C:\VOYAGER\DATA\MAG100\DIGEST\SMOOTH.MS Collected: 1/6/80 3:36 PM Sample: 42
Counts
Mass (m/z)
1297.51
1452.45
2073.84
1311.36
1397.52
2202.07
2668.48
1101.31
1127.42
1802.96
1855.64
3171.2
3200.16
1697.02
2045.78
1836.12
4321.35
Comment: barnes-20, tryp, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.090 %
Delay: 50 ON
Laser: 1820
Scans Averaged: 256
Pressure: 9.21e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.080
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7L

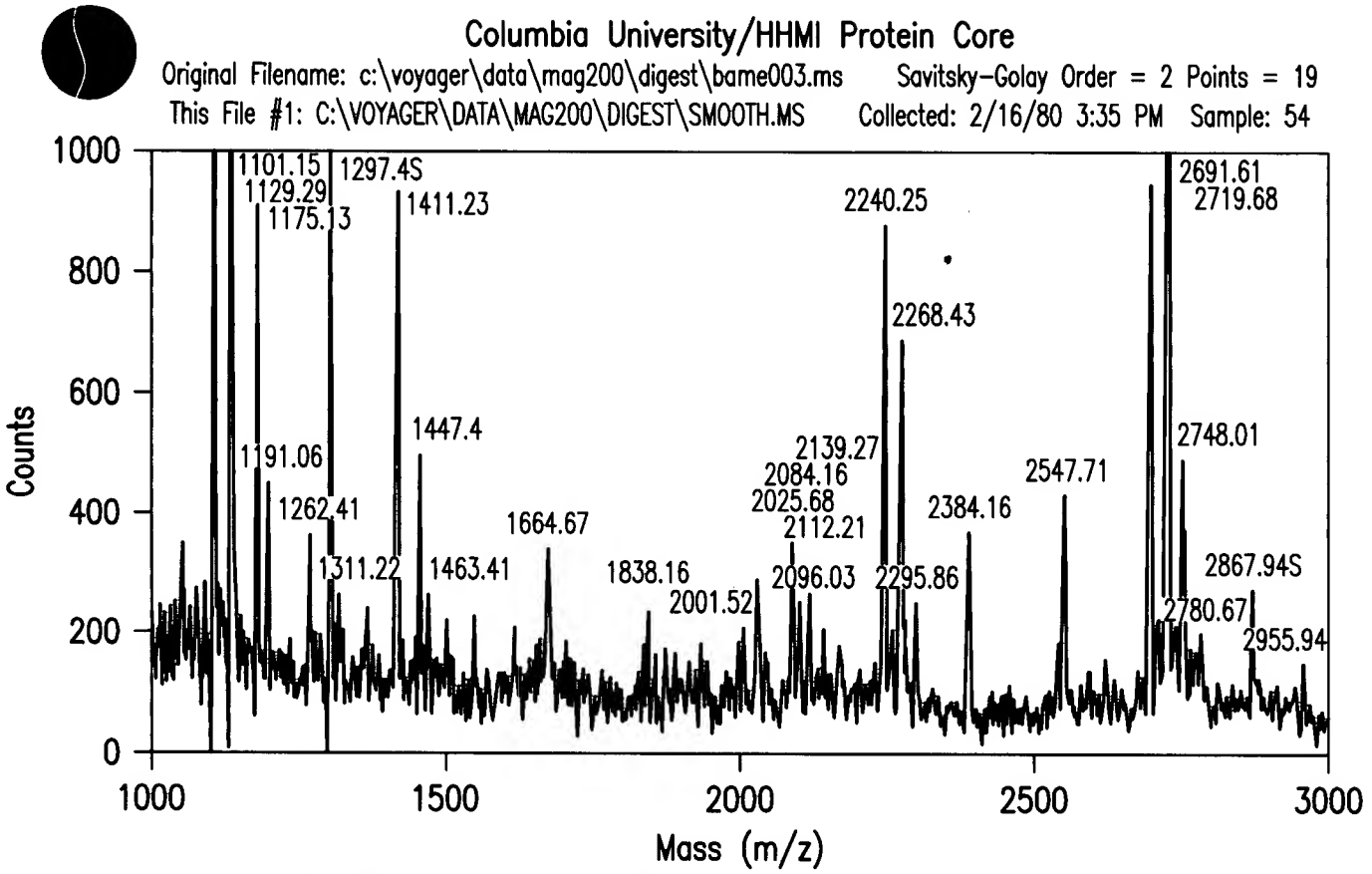

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag200\digest\barne003.ms Savitsky-Golay Order = 2 Points = 19
This File #1: C:\VOYAGER\DATA\MAG200\DIGEST\SMOOTH.MS Collected: 2/16/80 3:35 PM Sample: 54
Counts
Mass (m/z)
1000
800
600
400
200
0
1000
1500
2000
2500
3000
1101.15
1129.29
1175.13
1297.4S
1411.23
2240.25
2691.61
2719.68
2268.43
1447.4
1191.06
1262.41
2139.27
2084.16
2025.68
2112.21
2547.71
2748.01
2384.16
1664.67
1311.22
1463.41
1838.16
2001.52
2096.03
2295.86
2867.94S
2780.67
2955.94
Comment: barnes-22, mod.tryp., 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.090 %
Delay: 50 ON
Laser: 1870
Scans Averaged: 231
Pressure: 3.43e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.080
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG. 7M

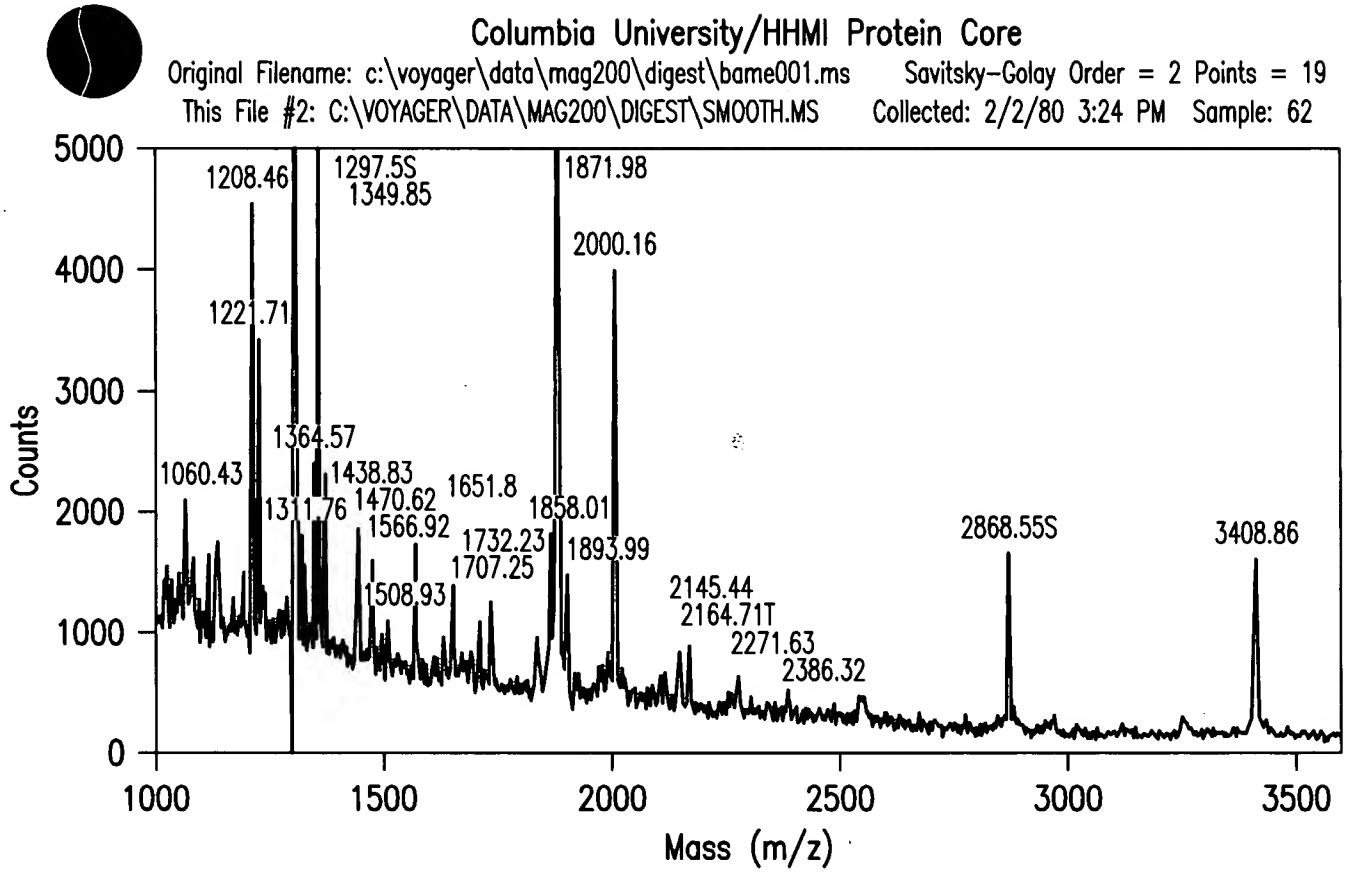

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag200\digest\barne001.ms Savitsky-Golay Order = 2 Points = 19
This File #2: C:\VOYAGER\DATA\MAG200\DIGEST\SMOOTH.MS Collected: 2/2/80 3:24 PM Sample: 62
Counts
Mass (m/z)
1208.46
1297.5S
1349.85
1871.98
2000.16
1221.71
1364.57
1060.43
1438.83
1470.62
1651.8
1311.76
1566.92
1858.01
1732.23
1893.99
1707.25
1508.93
2145.44
2164.71T
2271.63
2386.32
2868.55S
3408.86
Comment: barnes-25, tryp. modified, 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.090 %
Delay: 50 ON
Laser: 1860
Scans Averaged: 256
Pressure: 9.26e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.080
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7N

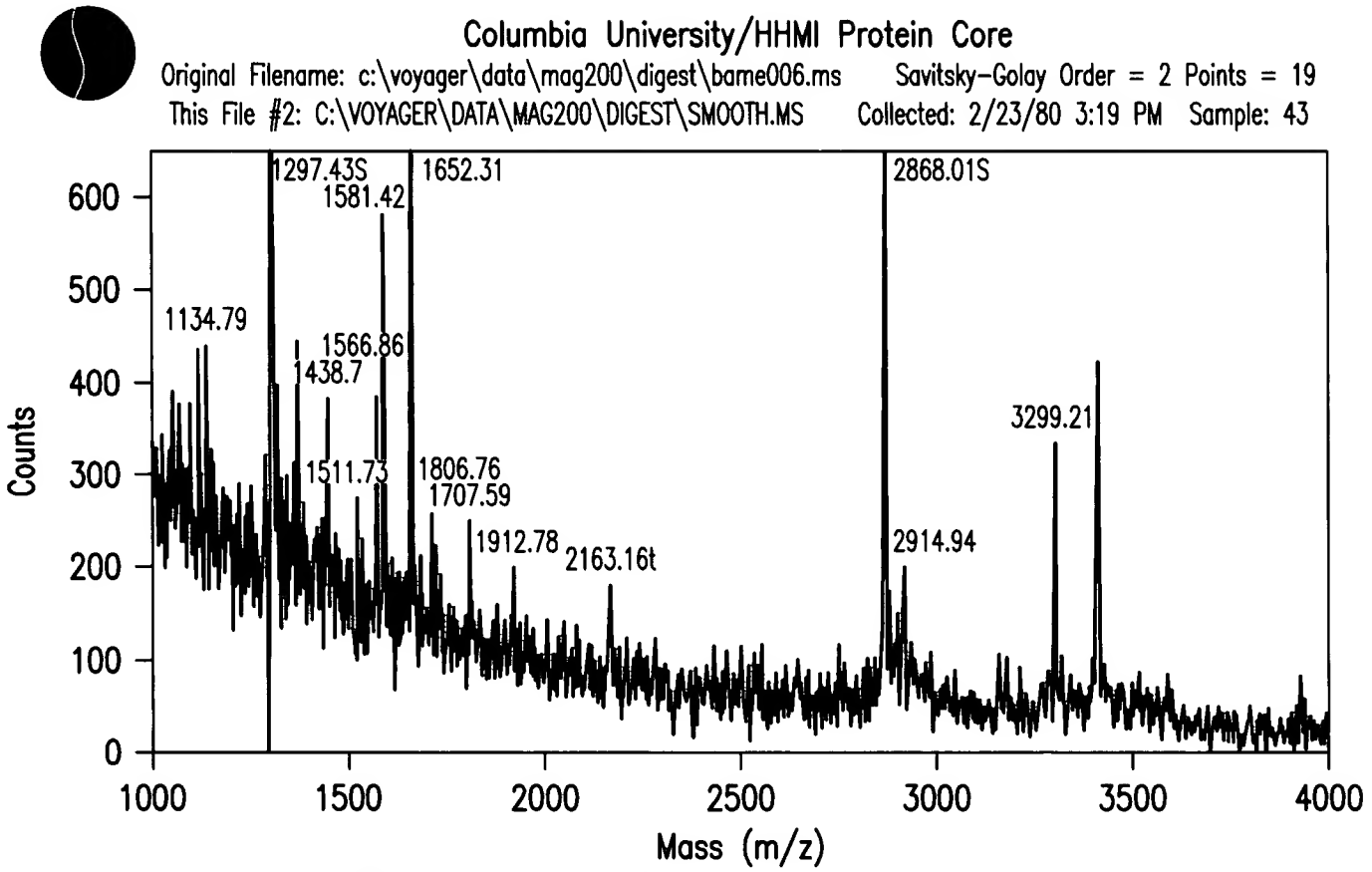

Columbia University/HHMI Protein Core
Original Filename: c:\voyager\data\mag200\digest\barne006.ms Savitsky-Golay Order = 2 Points = 19
This File #2: C:\VOYAGER\DATA\MAG200\DIGEST\SMOOTH.MS Collected: 2/23/80 3:19 PM Sample: 43
Counts
Mass (m/z)
1297.43S
1581.42
1652.31
2868.01S
1134.79
1566.86
1438.7
1511.73
1806.76
1707.59
1912.78
2163.16t
2914.94
3299.21
Comment: barnes-29, tryp.mod., 7.5 %
Method: LDE1000A
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.090 %
Delay: 50 ON
Laser: 1860
Scans Averaged: 256
Pressure: 3.82e-07
Low Mass Gate: 500.0
Mirror Ratio: 1.080
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative Ions: OFF

FIG.7O

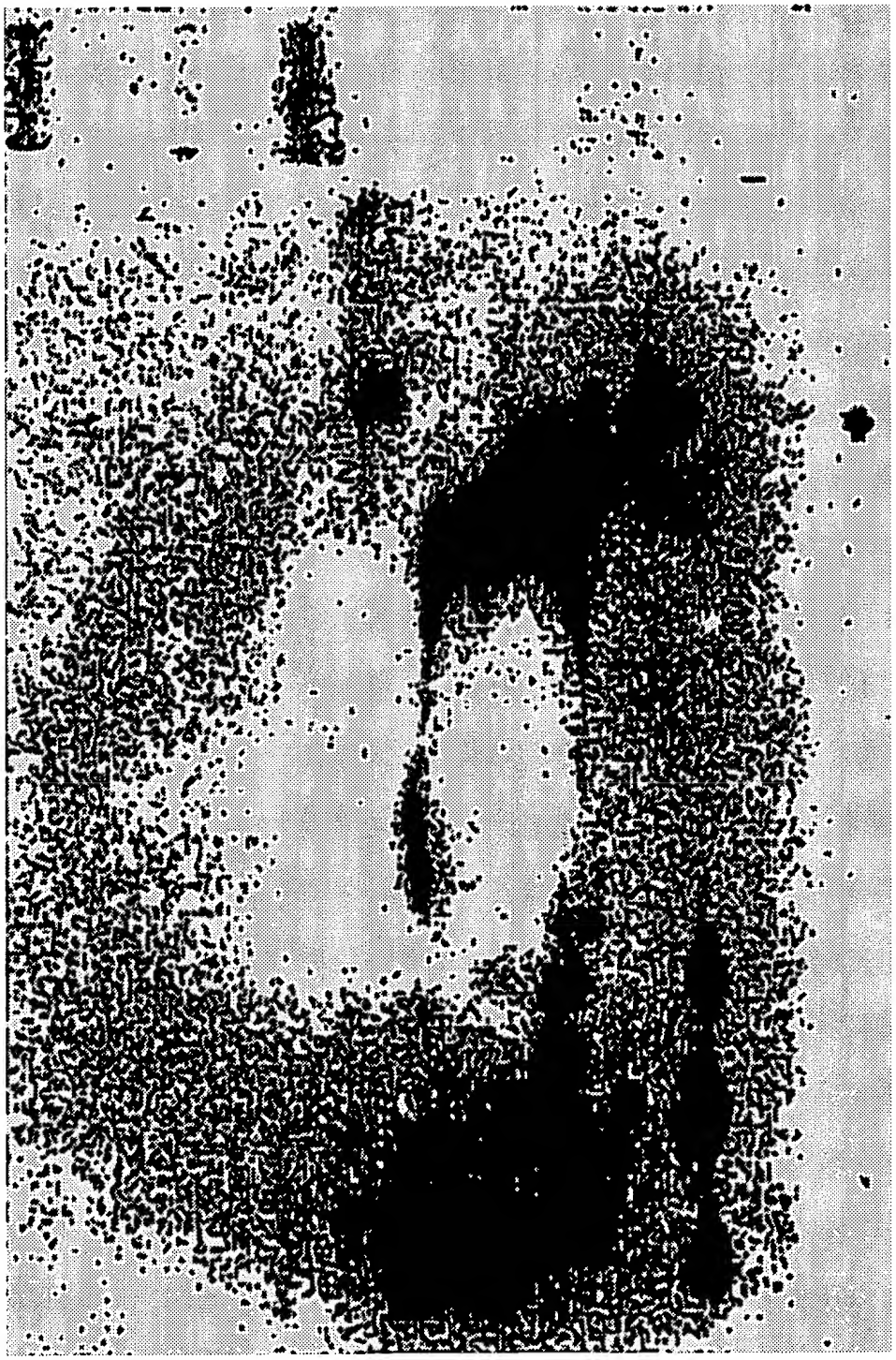


FIG.8

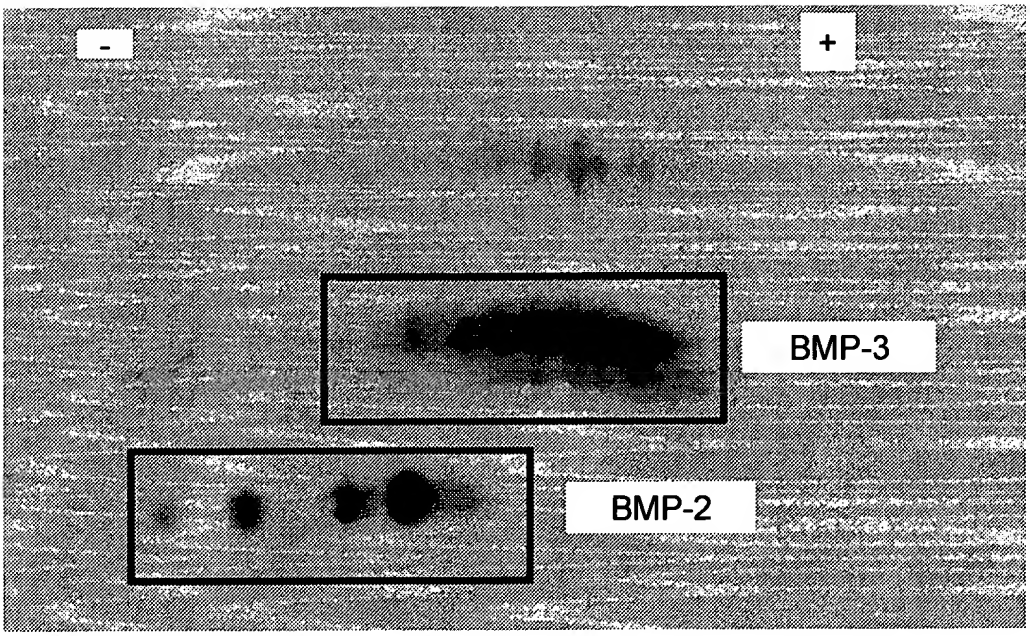


FIG.9A

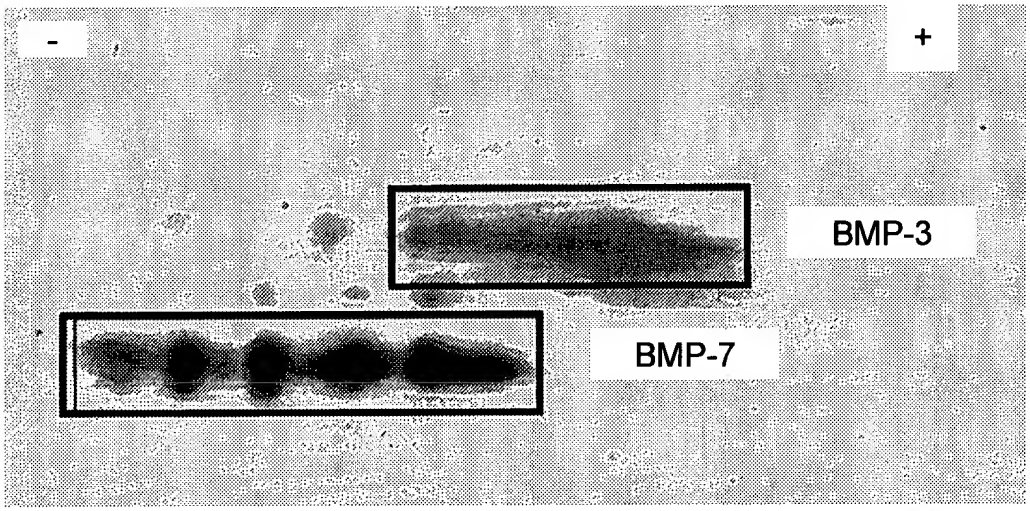


FIG.9B

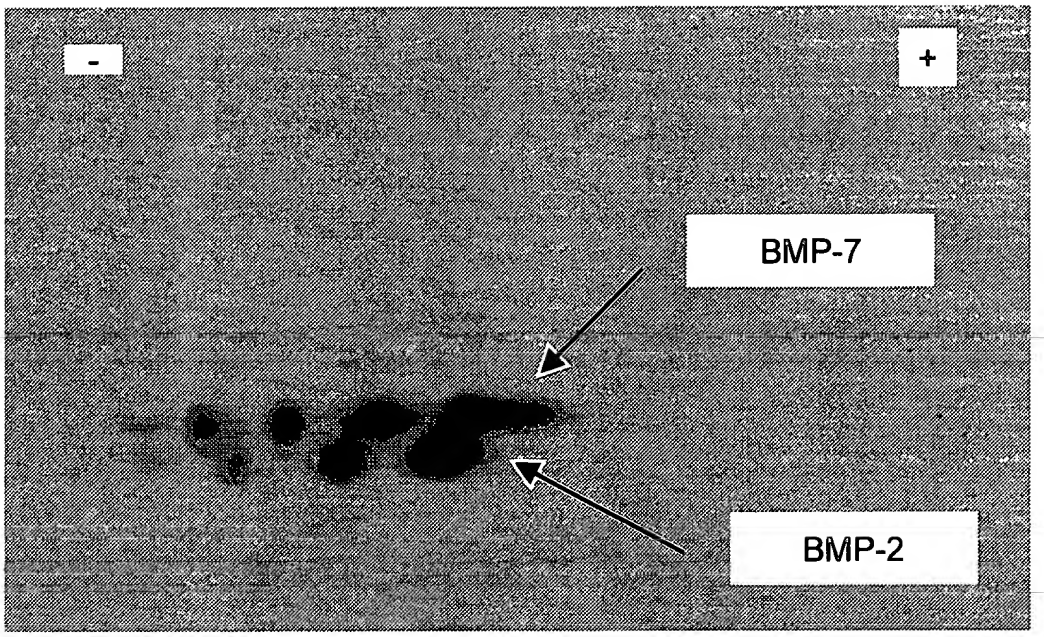


FIG.9C

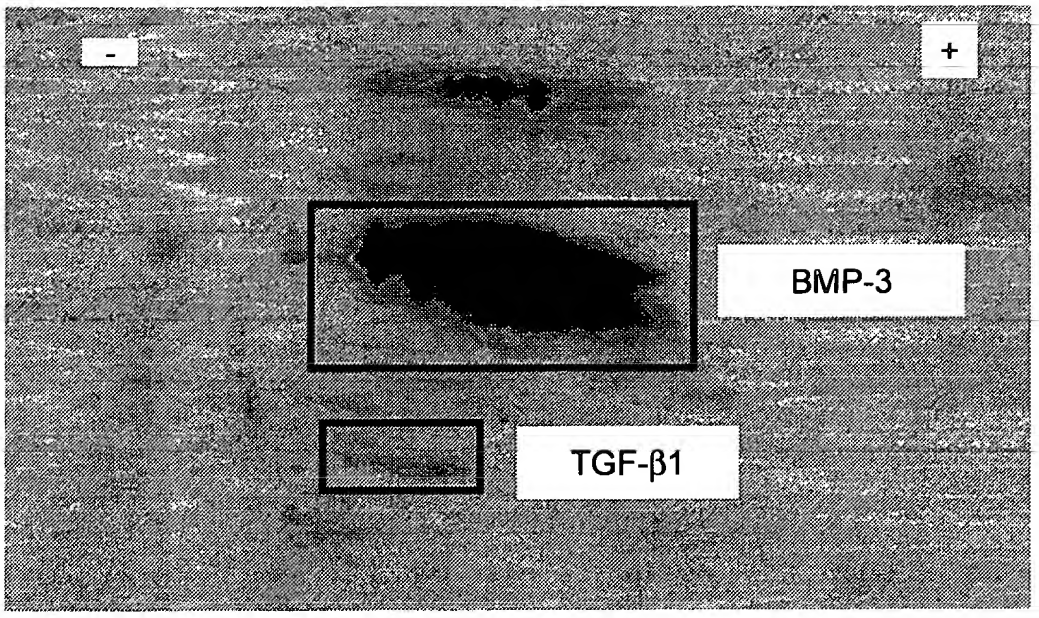


FIG.9D

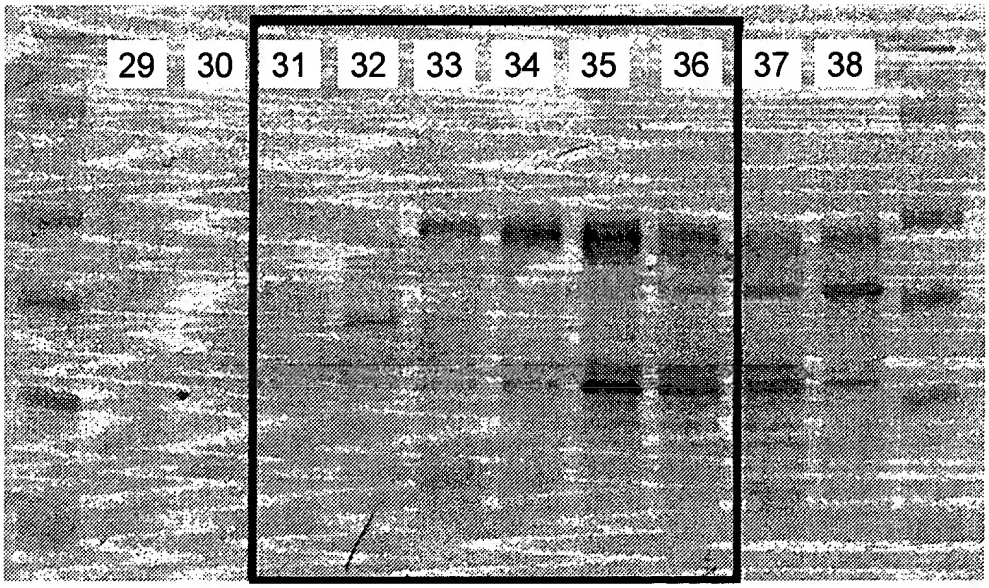


FIG.10

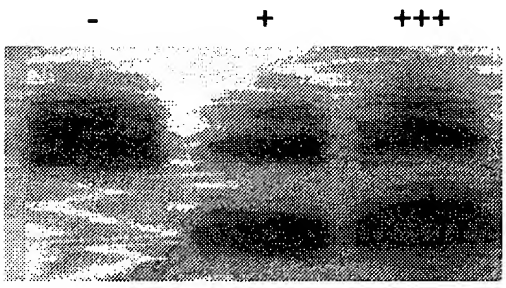


FIG.11

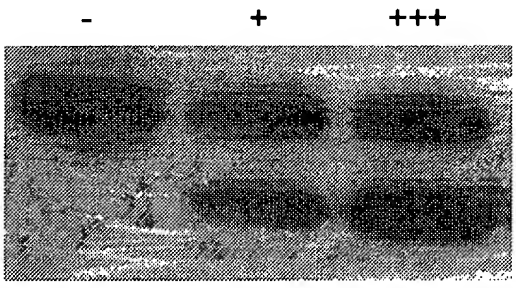


FIG.12

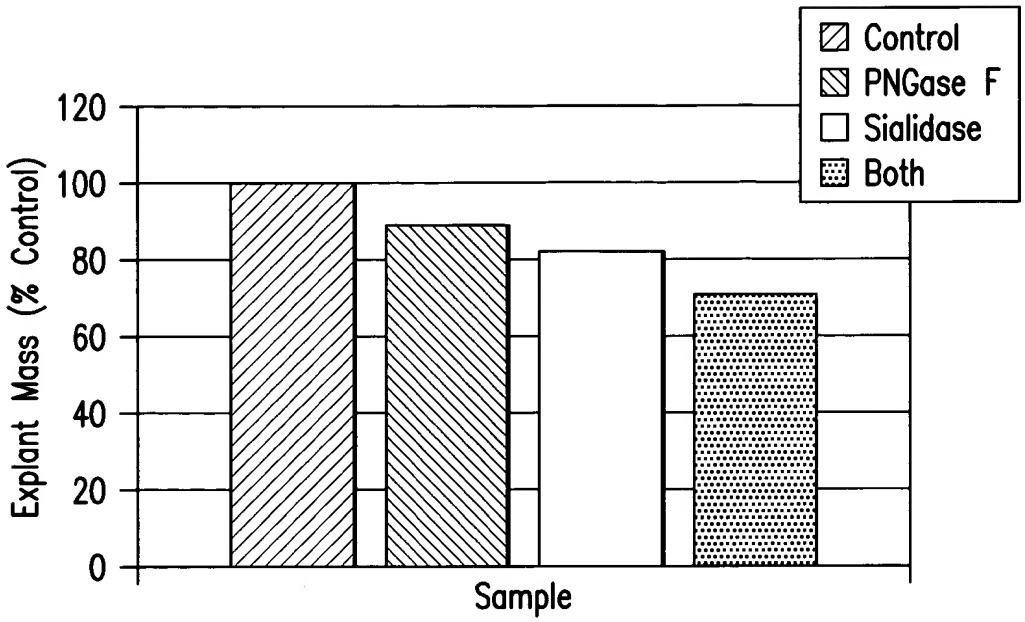


FIG.13A

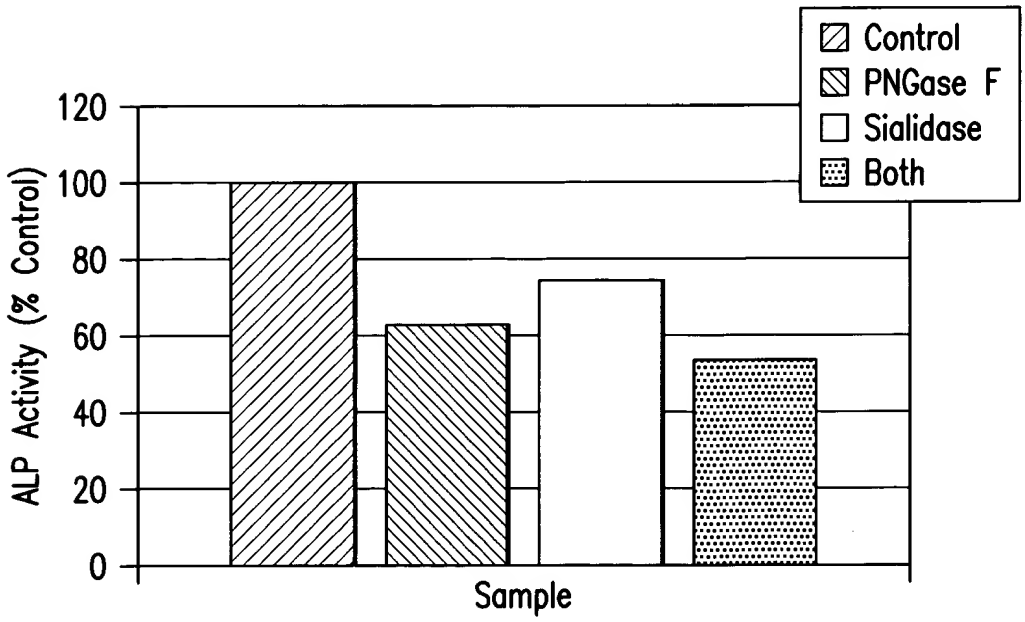


FIG.13B

Antibody Listing

Specificity	Antigen	Host Species	PC/MC	Source	Caralog No.
TGF-β1 (human)	Protein	Rabbit	Polyclonal	Promega	G1221
TGF-β2 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-90
TGF-β3 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-82
BMP-2 (human)	Protein	Rabbit	Polyclonal	Austral Biologics	PA-513-9
BMP-3 (human)	Peptide	Chicken	Polyclonal	Research Genetics	NA
BMP-4 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	so-6896
BMP-5 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-7405
BMP-6 (human)	Peptide	Mouse	Monoclonal	Novacastra Laboratories	NCL-BMP6
BMP7 (human)	Peptide	Rabbit	Polyclonal	Research Genetics	NA
FGF-1 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-1884
osteonectin (bovine)	Protein	Mouse	Polyclonal	DSHB	AON-1
osteocalcin (bovine)	Protein	Rabbit	Polyclonal	Accurate Chemicals	A761/R1H
serum albumin (bovine)	Protein	Rabbit	Polyclonal	Chemicon International	AB870
transferrin (human)	Protein	Chicken	Polyclonal	Chemicon International	AB797
apo-A1 lipoprotein (human)	Protein	Goat	Polyclonal	Chemicon International	AB740

FIG.14

Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Accession No.	AAs
1								
2	fx 49 (1579)	XLAAAGYDVEK	ALAAAGYDVEK	11/11	histone H1.c	human	87668 (NCBI)	65-75
3	fx 67 (1346)	SLEKVCADLIR	SLEKVCADLIR	11/11	40s Ribosomal Protein S20	rat	R3RT20 (PIR)	31-41
4	fx 65 ()	(V)VCGMLGFPSEAPV	VVCGMLGFPGEKRV	11/14	LORP	mouse	AAC95338 (NCBI)	213-226
5	N terminal seq	STGVLLPLQNNELPG	STGVLLPLQNNELPG	15/15	BMP-3	human	4557371 (NCBI)	290-304
	fx 72 (3925)	STGVLLPLQNNELPGAEYQY	STGVLLPLQNNELPGAEYQY	20/20	BMP-3	human	4557371 (NCBI)	290-309
	fx 74 (3409)	STGVLLPLQ	STGVLLPLQ	9/9	BMP-3	human	4557371 (NCBI)	290-298
6	fx 55 (1566)	(S)QTLQFXE	SQTLQFDE	7/8	BMP-3	human	4557371 (NCBI)	346-353
	fx 47	VYAF	no match		???			
	N terminal seq	HAGKYSREKNT(P)A(P)	HGGKYSREKNQPKP	11/14	α2-Macroglobulin Receptor Assoc. Pro.	human	P30533 (Swiss-Prot)	31-46
	fx 57 (1438)	SQTLQFDEQ	SQTLQFDEQ	9/9	BMP-3	human	4557371 (NCBI)	346-354
	fx 57 (1652)	SLKPSNHA	SLKPSNHA	8/8	BMP-3	human	4557371 (NCBI)	410-417
7	fx 51 (1093)	AALRPLVKP	AALRPLVKP	9/9	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	1-9
	fx 37 (no MS)	A(H)I(Q)VERYV	AIVER	5/5	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	109-113
	fx 37 (no MS)	A(H)I(Q)VERYV	HQSDRYV	5/7	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	22-28
8	fx 78 ()	XALF(G)AQLGXALGPI	no match		???			
9	fx 56 (1567)	SQTLQFDEQT	SQTLQFDEQT	10/10	BMP-3	human	P12645 (Swiss-Prot)	346-355

FIG.15A

Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Accession No.	AAs
11	fx 55 (1311)	SQTLXF	SQTLQF	5/6	BMP-3	human	4557371 (NCBI)	346-351
	fx 47 (1772)	VLATVTKPVGGDK	VLATVTKPVGGDK	13/13	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	87-99
	fx 76 (1795)	xVFAL	VFAL	4/4	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	273-276
	fx 61 (1145)	AVPQLQGYLR	AIPQLQGYLR	9/10	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	262-271
18								
22	fx 58 (1101)	ALDAAYCFR	ALDAAYCFR	9/9	TGF-β2	human	P08112 (Swiss-Prot)	303-311
	fx 69 (no match)	GYNANFCAGACPYL	GYNANFCAGACPYL	14/14	TGF-β2	human	P08112 (Swiss-Prot)	340-353
	fx 66 (1411.71)	VNSQSLSPY	VNSQSLSPY	9/9	SPP24	bovine	Q27967 (Swiss-Prot)	42-50
25	fx 39(1470)	KAAKPSV(P)	KAAKPSVP	8/8	histone H1 x	human	JC4928 (PIR)	199-206
29								

fx=fraction number (molecular weight of fragment, as measured by SDS-PAGE)

FIG.15B

Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
1	4 peaks match with histone H1.c	human	87668 (NCBI)	1172.97	1172.37	0.60	110–121	22	15 MS peaks match with Band 2
				1579.87	1579.71	0.16	65–79		
				1708.47	1707.89	0.58	64–79		
				2011.58	2012.32	−0.74	35–54		
2	3 peaks match with histone H1.c	human	87668 (NCBI)	1579.76	1579.71	0.05	65–79*	16	identification of starred peptide confirmed by sequence analysis
				1708.02	1707.89	0.13	64–79		
				2012.12	2012.32	−0.20	35–54		15 MS peaks match with Band 1
3	7 peaks match with ribosome S20	rat	R3RT20 (PIR)	1129.76	1129.40	0.36	50–59	62	
				1156.21	1156.30	−0.09	76–83		
				1334.46	1334.62	−0.16	56–66		
				1352.13	1351.58	0.55	88–99		
				1518.04	1517.77	0.27	9–21		
				1919.02	1919.19	−0.17	5–21		
				3404.02	3404.87	−0.85	88–119		
4	3 peaks match with Lysyl Oxidase RP	human	NP002309 (Swiss–Prot)	1987.95	1988.27	−0.32	150–167	6	12 MS peaks match with Band 8
				2410.35	2410.63	−0.28	648–669		
				2610.57	2610.10	0.47	455–478		

FIG.16A

Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs *	% Coverage	Comments
5	9 peaks match with BMP-3	human	4557371 (NCBI)	1113.32	1113.31	0.01	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1438.53	1438.58	-0.05	346-357		
				1566.76	1566.76	0.00	345-357		
				1651.86	1651.91	-0.05	410-424		
				1794.09	1794.02	0.07	346-360		
				2268.46	2268.63	-0.17	374-392		
				2424.45	2424.81	-0.36	373-392		identification of starred peptide confirmed by sequence analysis
				3409.15	3407.77	1.38	290-318*		
6	3 peaks match with α2-Macroglobulin RAP	human	P30533 (Swiss-Prot)	1002.24	1002.15	0.09	283-290	17	
				2362.58	2362.43	0.15	129-150		
				3048.51	3048.52	-0.01	257-282		
	2 peaks match with BMP-3	human	4557371 (NCBI)	1566.93	1566.75	0.18	346-357	15	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1651.88	1651.91	-0.03	410-424		

FIG.16B

Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
7	4 peaks match with ribosome L32	mouse	P17932 (Swiss-Prot)	1033.25	1033.17	0.08	67-75	33	
				1093.31	1093.40	-0.09	1-10*		
				1134.72	1134.28	0.44	65-74		
				1449.78	1449.66	0.12	19-29		
	5 peaks match with BMP-3	human	4557371 (NCBI)	1060.42	1060.20	0.22	102-111	21	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1113.39	1113.31	0.08	361-368		
				1360.26	1360.58	-0.32	190-200		
				1652.28	1651.91	0.37	410-424		
				1793.62	1794.02	-0.40	346-360		
8	1 peak match with Lysyl Oxidase RP	human	NP002309 (Swiss-Prot)	2410.37	2410.63	-0.26	648-669	3	12 MS peaks match with Band 4
9	6 peaks match with BMP-3	human	4557371 (NCBI)	1113.14	1113.31	-0.17	361-368	36	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1438.60	1438.58	0.02	346-357		
				1566.77	1566.76	0.01	345-357		
				1651.91	1651.61	0.30	410-424		
				2901.67	2901.19	0.48	41-66		
				3408.94	3407.77	1.17	290-318		

FIG.16C

Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
11	5 peaks match with BMP-3	human	4557371 (NCBI)	1113.23	1113.31	-0.08	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1651.73	1651.91	-0.18	410-424		
				1793.58	1794.02	-0.44	346-360		
				2424.24	2424.81	-0.57	373-392		
				3408.34	3407.77	0.57	290-318		
	5 peaks match with ribosome L6	human	Q02878 (Swiss-Prot)	1140.38	1140.23	0.15	114-122	16	
				1526.88	1526.86	0.02	141-155		
		mouse	P47911 (Swiss-Prot)	1059.15	1059.12	0.03	10-20		
				1145.36	1145.35	0.01	262-271		
				1386.74	1386.68	0.06	260-271		
18	4 peaks match with TGF-β2	human	P08112 (Swiss-Prot)	1101.20	1101.26	-0.06	303-311	52	
				1175.26	1175.42	-0.16	400-409		
				2240.37	2240.60	-0.23	312-328		
				2691.70	2691.91	-0.21	340-362		
	5 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	1410.93	1411.60	-0.67	42-53	30	
				1447.59	1447.65	-0.06	113-124		
				1540.64	1540.60	0.04	86-98		
				1869.10	1869.05	0.05	62-77		
				2268.47	2268.57	-0.10	33-53		

FIG.16D

Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
22	5 peaks match with TGF-β2	human	P08112 (Swiss-Prot)	1101.15	1101.26	-0.11	303-311	63	
				1175.13	1175.42	-0.29	400-409		
				2084.16	2084.42	-0.26	312-347		
				2240.25	2240.60	-0.35	312-328		
				2691.61	2691.91	-0.30	340-362		
	2 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	1411.23	1411.60	-0.37	42-53	11	
				1447.40	1447.65	-0.25	113-124		
25	5 peaks match with histone H1.x	human	JC4928 (PIR)	1208.46	1208.40	0.06	48-57	14	
				1221.71	1222.35	-0.64	107-118		
				1349.85	1350.52	-0.67	107-119		
				1364.57	1364.59	-0.02	48-58		
				1732.23	1732.97	-0.74	43-57		
	5 peaks match with BMP-3	human	4557371 (NCBI)	1060.43	1060.20	0.23	102-111	31	% coverage calculation is relative to the mature BMP-3, 183 AAS (280-472)
				1438.83	1438.58	0.25	346-357		
				1566.92	1566.76	0.16	345-357		
				1651.80	1651.91	-0.11	410-424		
				3408.86	3407.77	1.09	290-318		

FIG.16E

Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
29	4 peaks match with BMP-3	human	4557371 (NCBI)	1113.22	1113.31	-0.09	361-368	27	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1438.70	1438.58	0.12	346-357		
				1566.86	1566.75	0.11	345-357		
				3409.04	3407.77	1.27	290-318		

FIG.16F

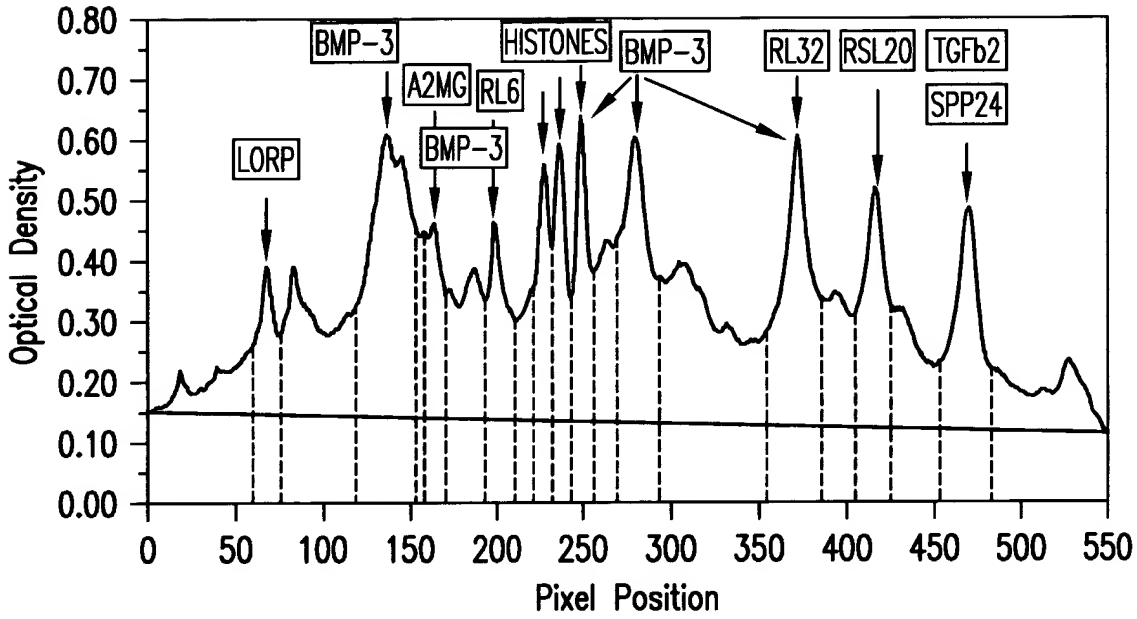


FIG.17A

FIG.17B

Quantitation of Identified BP proteins

Identified protein	Percentage of Total Protein
LORP	2
BMP-3	11
BMP-3 and A2-MG	3
RL6 & BMP-3	4
Histone	3
Histone	3
Histone & BMP-3	4
BMP-3	8
RL32 & BMP-3	8
RS2D	5
SPP24 & TGF-β2	6
Total	58%

FIG.18

Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spal	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
1	Lys-C	2 peaks match with Coagulation Factor XINb	Human	P05160 (Swiss-Prot)	1837.01	1637.14	-0.13	472-437	5	
					1921.85	1921.14	0.51	368-982		
					2679.51	N/A	N/A	489-504		peptide match confirmed by sequence analysis
2	Trypsin	2 peaks match with LORP	Human	NP002309 (Swiss-Prot)	1609.57	1609.68	-0.31	241-253	5	
					2410.89	2410.63	0.28	445-669		
3	Lys-C	5 peaks match with Cathepsin L Precursor	Bovine	P25075 (Swiss-Prot)	1407.26	1406.60	9.40	105-118	41	
					1546.64	1548.70	0.14	58-70		
					1681.18	1660.80	0.36	21-33		
					1881.46	1680.80	-1.06	301-314		
					1834.71	1634.00	0.71	318-334		
					2352.90	2351.50	1.40	274-295		
					2381.50	2380.70	12.50	239-251		
					2721.51	2721.10	12.41	131-154		

FIG.19A

Identifications of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spal	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
7	Lys-C	4 peaks match with TGF-β2	Bovine	P21214 (Swiss-Prot)	774.56	774.80	-0.34	26.31	42	
					809.69	809.94	-0.25	32.37		
					1175.12	1175.43	-0.31	88.107		
					3168.10	3166.66	1.44	1-25		
	Trypsin	1 peak matchs with SPP24	Bovine	Q27957 (Swiss-Prot)	2167.77	2167.51	0.26	42.60	10	
8	Trypsin	12 peaks match with Ribosome L3	Bovine	P38872 (Swiss-Prot)	917.39	917.14	0.25	348.355	37	
					984.23	984.15	0.08	10.18		
					1193.62	1192.40	0.22	286-296		
					1360.67	1360.65	0.02	249-250		
					1484.60	1484.63	0.17	103-114		
					1620.88	1620.82	0.04	103-115		
					1778.64	1777.00	-0.16	34-49		
					2238.43	2238.55	-0.12	30-49		
					2325.99	2325.65	0.34	177-197		
					2681.31	2681.04	0.27	200-223		
					2597.94	2597.43	-0.49	70-98		
					2946.10	2946.35	-0.25	198-223		

FIG.19B

Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spal	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
7	Lys-C	4 peaks match with TGF-β2	Bovine	P21214 (Swiss-Prot)	774.56	774.80	-0.34	26.31	42	
					809.69	809.94	-0.25	32-37		
					1175.12	1175.43	-0.31	88-107		
					3168.10	3166.66	1.44	1-25		
		1 peak matchs with SPP24	Bovine	Q27957 (Swiss-Prot)	2167.77	2167.51	0.26	42-60	10	
8	Trypsin	12 peaks match with Ribosome L3	Bovine	P38872 (Swiss-Prot)	917.39	917.14	0.25	348-355	37	
					984.23	984.15	0.08	10-18		
					1192.62	1192.40	0.22	286-296		
					1360.67	1360.65	0.02	249-250		
					1484.60	1484.63	0.17	103-114		
					1620.88	1620.82	0.04	103-115		
					1778.64	1777.00	-0.16	34-49		
					2238.43	2238.55	-0.12	30-49		
					2325.99	2325.65	0.34	177-197		
					2681.31	2681.04	0.27	200-223		
					2597.94	2597.43	-0.49	70-98		
					2946.10	2946.35	-0.25	198-223		

FIG.19C

Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spal	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
9	Trypsin	7 peaks match with Ribosome S3	Mouse	P97351 (Swiss-Prot)	920.05	820.10	−0.05	19−25	29	
					1218.29	1218.31	−0.02	152−181		
					1346.82	1348.49	0.13	151−161		
					1516.69	1516.69	0.00	174−186		
					1593.72	1593.82	−0.10	94−108		
					1719.91	1720.08	−0.09	199−212		
					1953.12	1953.16	−0.04	65−81		
10	Trypsin	4 peaks match with histone H1.c	Human	07658 (NCBI)	1327.75	1327.58	0.19	34−46	23	
					1579.70	1579.71	−0.01	65−78		
					1707.65	1707.89	−0.24	64−79		
					2147.17	2147.53	−0.36	1−21		
11	Trypsin	6 peaks match with Ribosome S4	Human	P12750 (Swiss-Prot)	1188.48	1168.38	0.10	230−239	23	
					1216.39	1216.39	0.00	134−144		
					1354.09	1353.61	0.42	230−241		
					1507.81	1507.89	0.12	108−210		
					1557.75	1557.98	−0.23	37−48		
					2140.34	2140.58	−0.24	221−239		
					2591.80	2591.90	−0.10	77−98		

FIG.19D

Quail Chorioallantoic Membrane (CAM) Angiogenesis Assay

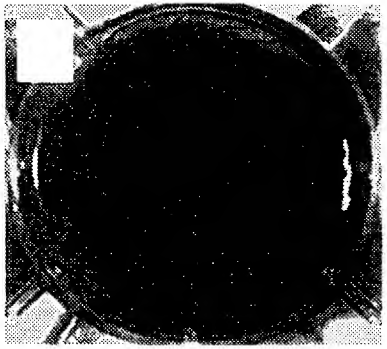


FIG.20A

Quail Chorioallantoic Membrane (CAM) Angiogenesis Assay

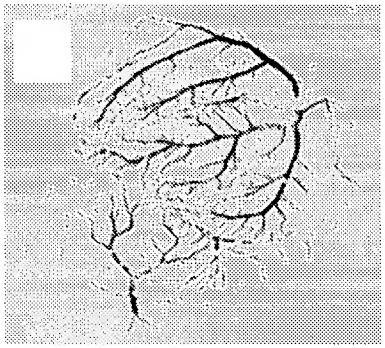


FIG.20B

Quail Chorioallantoic Membrane (CAM) Angiogenesis Assay

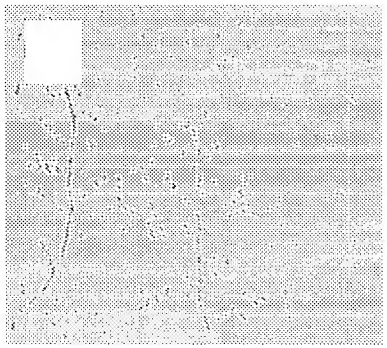
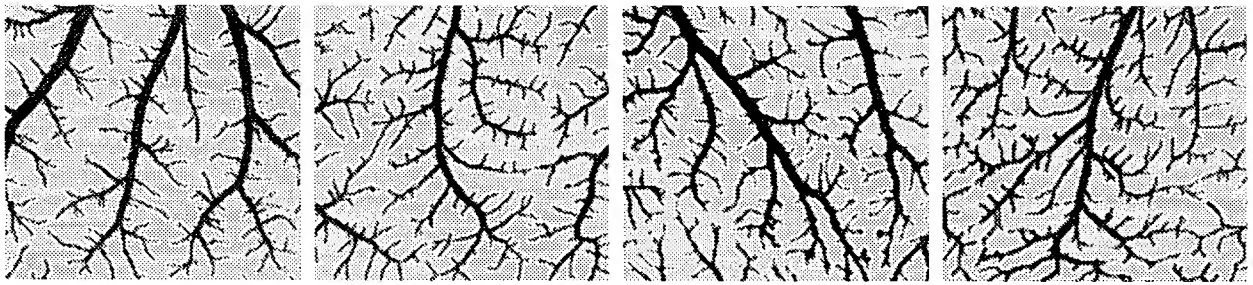


FIG.20C

Black and white images of CAM vasculature after growth factor treatment



PBS
100 %

10 µg/ml bFGF
145 %

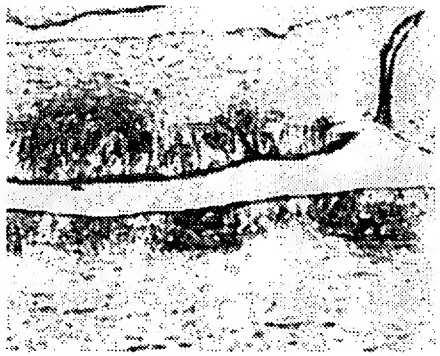
10 µg/ml BP
224 %

10 µg/ml IBP
186 %

FIG.21

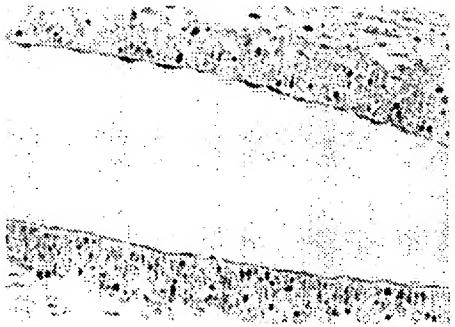
Histological sections of blood vessels formed in canine myocardium 2 weeks following BP injection

A. Trichrome stain

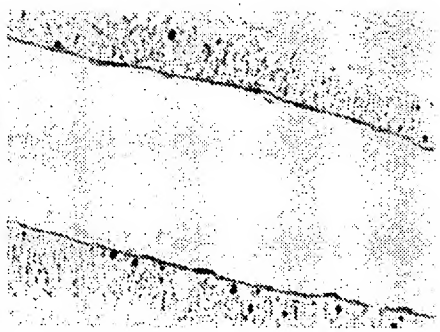


B. Factor VIII

C. Smooth Muscle Actin (SMA)



D. Proliferating Cell Nuclcar Antigen (PCNA)



E. BrdU

FIG.22

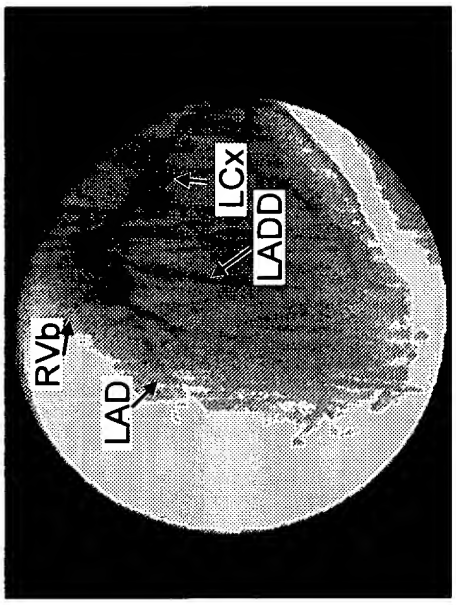


FIG.24

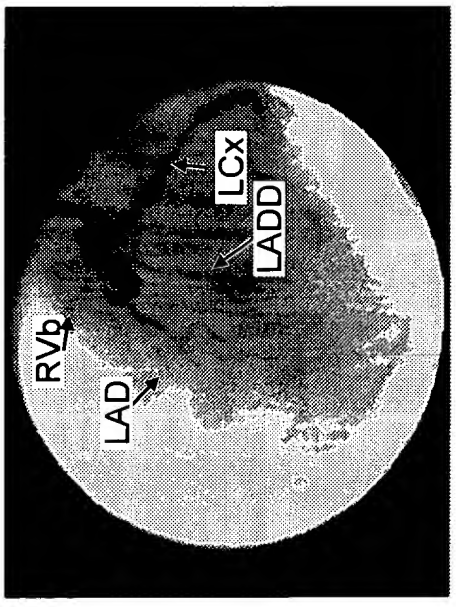


FIG.23

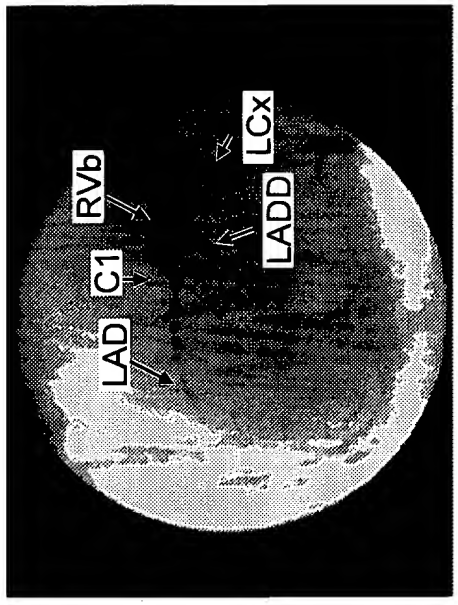


FIG.26

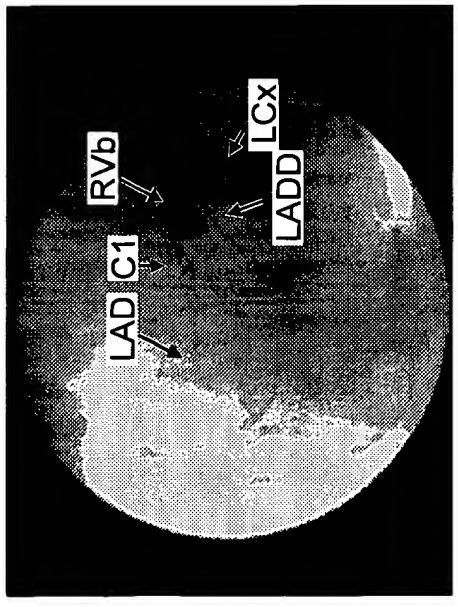


FIG.25